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INTERESTING SPECIES OF LACTARIAE FROM FLORIDA

H. C. BEARDSLEE AND GERTRUDE S. BURLINGHAM

(WITH 4 FIGURES)

The following species of Lactariae have been collected by the authors for several fall and winter seasons in various localities in Orange and Seminole counties, Florida.

Lactaria floridana sp. nov. (FIG. 1, *A*; 4, *A*)

Pileus fleshy, firm, umbilicate with arched incurved margin, at length spreading and deeply depressed in the center, up to 12 cm. broad; surface varying from brownish terra cotta [1] to cinnamon or buff or pale buff with the center ochraceous to maize yellow, azonate or sometimes faintly zoned, very viscid when young even in dry weather, tomentose on the margin and half way to the center or sometimes up to the disc with long tangled agglutinated fibres which do not project beyond the margin as in *Lactaria torminosa;* context firm, thick, white to pale yellowish flesh, aromatic with pleasant odor remaining when dried; latex white, unchanging, scanty, very acrid; lamellae nearly white at first, becoming pale ecru tone 4 singly, then near honey-yellow tone 1, and finally ochroleucous on the edges, and isabelline tone 1 in position, unequal, mostly simple with a few forking near the stipe, broad in the outer half, narrowed toward the stipe and attached with a decurrent tooth, close; stipe pale blush to cinnamon tone 1 or tinted with it, becoming maize tone 4 with age, pruinose at the apex, sometimes with small scrobiculate spots at base or on the

[1] Unless otherwise noted the colors used are those of the Repertoire de Couleurs.

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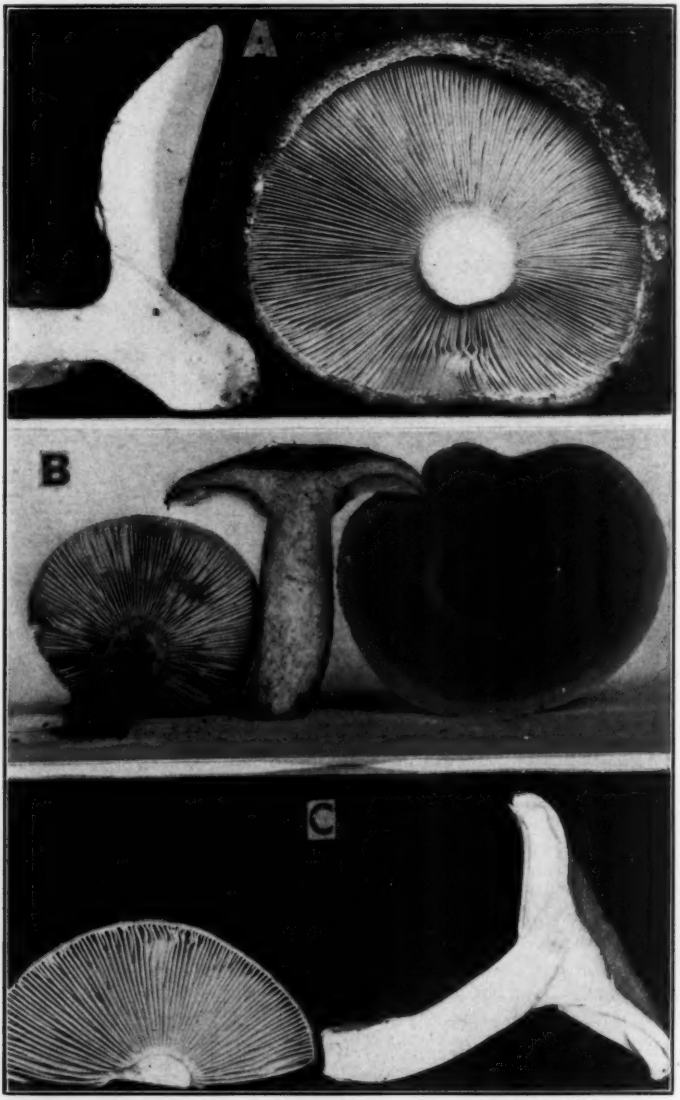


FIG. 1. *A, Lactaria floridana* × 1, often larger; *B, Lactaria cognoscibilis* × 1; *C, Lactaria impercepta* × 1.

lower half, nearly equal, from 2–4 cm. × 1.4–2.3 cm., firm, s 1; spores pale blush tone 1–2 to pale yellowish flesh tone 2 in thick mass, 5–5.5–6.25 μ × 6.87–7.5–8.75 μ, uniguttulate and apparently smooth under the 1/6 power without iodine, but under oil immersion with iodine they are seen to be reticulate with scattered protuberances, unsymmetrical, apiculate.

Pileo carnoso, firmo, umbilicato, margine arcuato et incurvato, demum expanso et centro profunde depresso, colore variabili, e brunneo-latericeo (322) cinnamoneo (323) aut isabellino (309) et centro ochraceo, azono aut leviter zonato, primo viscossisimo etiam siccitate, margine aut ad medium fibris longis agglutinatisque tomentosis sed non ut in *Lactaria torminosa* exstantibus; carne firma, crassa, alba, pallide lutescente, odoro grato aromaticoque etiam siccata; lacte albo, immutabili, exiguo, acerrimo; lamellis primo subalbis demum pallide lutescentibus (66 t-4), simplicibus aut paucis ad stipitem furcatis; stipite e carneo-albo (137) cinnamoneo, apice pruinoso, basi interdum leviter scrobiculato, subaequali; sporis e incarnato-albis (137 t 1–2) luteolo-albis (68 t-2), 5–5.5–6.25 μ × 6.87–7.5–8.75 μ, uniguttulatis, minute echinulatis et lineis delicatis reticulatis.

TYPE LOCALITY: On Route 24 about three miles north of Apopka, Orange Co., Florida.

HABITAT: In sandy soil in rather open live oak and pine woods, Dec. and Jan.

This species differs from *Lactaria scrobiculata* (Scop.) Fries in its unchanging latex and scarcely zonate pileus; from *Lactaria torminosa* (Schaeff.) Pers. it differs in the character of the tomentum on the margin which is agglutinated on the surface and does not form a fringe on the edge and in the very small protuberances on the spores. Also in the coloration both in the field and when dried it does not resemble that species. Some specimens were found in places which had been lightly burned over a few months before.

Lactaria cognoscibilis sp. nov. (FIG. 1, *B*; 4, *B*)

Pileus broadly convex becoming plane to a little centrally depressed, sometimes with a small sharp umbo, 3.5 to 8.5 cm. broad; surface mineral brown tone 1 or paler zoned with deeper tones, with a white pruinose bloom at first, dull, fading and becoming azonate, a little viscid when wet; margin arched but finally upturned and remaining pruinose for some time; context near dark fawn, decidedly peppery and lasting; latex white, unchanging, abundant, bitter then slowly peppery, more quickly and intensely peppery in young specimens; lamellae pale flesh tone 4 singly, unequal, sometimes forking near the stipe, adnate with a slightly de-

current tooth, interveined next the pileus, pruinose when mature, close; stipe paler than the pileus, pruinose, nearly equal but abruptly smaller at the base, solid 3–3.5 cm. × .7–1.2 cm.: spores fleshy-white tone 3 in thick mass, broadly elliptical to globose, coarsely echinulate with some connecting bands, apiculate, 7.5–8 × 8–8.75 μ or 8.1 × 8.75 μ or 8.75 × 8.75 μ.

Pileo e late convexo, expanso aut subdepresso, interdum acute subumbonato, 3.5–8.5 cm. lato, minerale-brunneo (339) aut pallidiore et zonis orscurioribus notato, demum pallidiore et azono, primo leniter albo-pruinoso, udo viscidulo, diu pruinoso; carne fusca fulva (307), diu acerrima; lacte albo, immutabili, abundante, amaro et tarde acri, aetate juniore acriore; lamellis pallide incarnatis (136 t-4) inaequalibus, paucis ad stipitem furcatis, adnatis et dente subdecurrentibus demum pruinosis, confertis; stipite pileo pallidiore, pruinoso, subaequali basi abrupte constricto, solido, 3–3.5 cm. × .7–1.2 cm.; sporis albidulis (9 t-3) late ellipticis aut subglobosis, valde echinulatis et reticulatis, apiculatis, 7–8 × 8–8.75 μ ad 8.7 × 8.7 μ.

TYPE LOCALITY: The Black Hammock, Oviedo, Florida.

HABITAT: In black sandy humus under sweet gum with live oaks and cabbage palms near, often gregarious.

DISTRIBUTION: Widely scattered in the type locality, also in a hammock near Green Springs, Volusia Co., Florida.

This species resembles *Lactaria mutabilis* Peck in color and zonation but can quickly be distinguished by the bitter peppery taste of the latex. It is common in the hammocks where found, from November through January when the weather is not too cold or dry. In large drops the latex seems to separate into milky and clear.

Lactaria proximella sp. nov. (FIG. 2, *A*; 4, *C*)

Pileus broadly convex-umbilicate, then expanding and shallowly infundibuliform, brownish-terra-cotta tone 2 zoned with tone 4, to cinnamon, whitish pruinose in the center, viscid when wet, 2.5 to 6 cm. broad; margin arched, at length uplifted and more or less fluted, sometimes striate; context paler than the lamellae but not pure white, odor none, wounds especially in the stipe may turn glaucous after some time where the latex has dried; latex white unchanging, scanty, very peppery; lamellae nearly white at first becoming chamois colored to cinnamon tone 1 singly, unequal, some forking near the stipe, broader than the flesh, slightly decurrent, close; stipe paler than the pileus, isabelline to maize tone 3–4, nearly equal except spreading a little at the apex, pruinose when young, 1.3–2 cm. × .8–1 cm.; spores fleshy white tone 2 to pale

blush tone 1–4, 7.5–8 $\mu \times 9.4\ \mu$, echinulate under the 1/6 power but with iodine stain and oil immersion the echinules are connected by bands, apiculate, unsymmetrical.

Pileo late convexo-umbilicato demum expanso et subinfundibuliformi, brunneo-latericeo (322 t-2) obscurioribus zonis notato, ad cinnamoneum, centro albo-pruinoso, udo viscido, 2.5 to 6 cm. lato; margine arcuato demum recurvato, flexuoso, interdum striato; carne lamellis pallidiore, inodora, vulnerata interdum glaucescenti; lacte albo, immutabili, exiguo, acerrimo: lamellis primo subalbis, tum e pallidis cinnamoneis, aequalibus, paucis ad stipitem furcatis, carne latioribus, subdecurrentibus, confertis; stipite pileo pallidiore, subaequali sed apice leniter incrassato, primo pruinoso, 1.3–2 cm. × .8–1 cm.; sporis albidulis (9 t-2), 7.5–8 $\mu \times 9.4\ \mu$, echinulatis et reticulatis.

TYPE LOCALITY: Oak woods on the shores of Lake Wildmere, Longwood, Florida.

HABITAT: Under black and live oaks in sandy soil, from November into January.

DISTRIBUTION: In various localities in the type woods, also under scrub oaks in the scrub near the Apopka airport, and in the hammock at the Fort Christmas Land Development Co., near Fort Christmas, Orange Co., Florida.

This species resembles *Lactaria insulsa* Fries in color but is much smaller, less zonate, and the wounds in the stipe often turn green, and the spores are smaller. From *Lactaria zonaria* Fries it differs in the less viscid scarcely zonate or azonate pileus, smaller size, lack of odor and the smaller spores. According to Rea the lamellae of *Lactaria zonaria* sometimes "become dingy or even somewhat aeruginous when bruised." The change of color in *Lactaria proximella* occurs chiefly on wounds of the stipe. From *Lactaria coleopteris* Coker it differs in lack of odor, less viscidity, lack of the white collar at the apex of the stipe, and the larger spores. Since this species is plainly rather closely related to all the species of this group, yet differs from each, the specific name seems appropriate.

Lactaria limacina sp. nov. (FIG. 2, *B, C;* 4, *E*)

Pileus umbilicate with inrolled margin becoming infundibuliform, up to 9 cm. broad; surface tinted snuff brown tone 1 when young, then tone 2 to putty color or chamois tone 1 when mature, azonate, slimy viscid with cuticle separable nearly one-half way to the center, glabrous to within about one cm. of the margin; margin

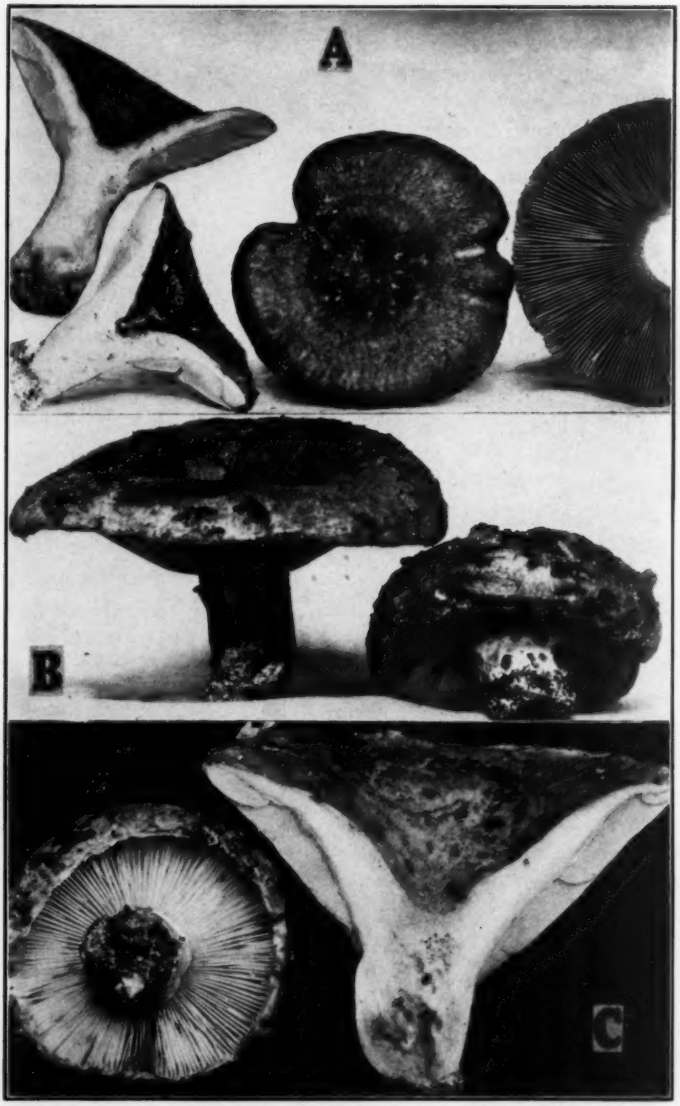


FIG. 2. *A, Lactaria proximella* × 1; *B, C, Lactaria limacina* × 1.

with agglutinated tangled tomentum with short fibres projecting from the involute edge; context pale yellowish flesh tone 1, or with a tint of snuff brown, odor none; latex white unchanging, slowly very peppery; lamellae whitish at first, becoming putty color singly and dark fawn in position, unequal, a few forking near the stipe, narrow at the inner end and slightly decurrent, close; stipe whitish at first then putty color, solid, sometimes scrobiculate, slimy, 1.7 to 2 cm. × 1 cm. to 1.8 cm. at the apex and 1.3 to .8 cm. at the base: spores fleshy-white tone 2 to 3, coarsely echinulate with echinules of different sizes connected by lines or bands of varying widths, apiculate, 8–8.75 μ × 10 μ.

Pileo umbilicato, margine incurvato, demum infundibuliformi, Havanna-brunneo (303 t-1) tum pallidiore, azono, viscoso, pellicula margine separabili, disco glabro sed margine involuto fibris agglutinatis projecantibusque tomentose; carne pallide luteo-incarnata (68 t-1), aut brunneo tincta, inodoro; lacte alba, immutabili, tarde acerrimo; lamellis albidis tum luteolis (311), inaequalibus, paucis ad stipitem furcatis, postice angustatis, subdecurrentibus, confertis; stipite ex albido luteola (311), solido interdum scrobiculato, viscoso, 1.7–2 cm. × 1–1.8 cm. apice, basi leniter constricto: sporis albidis (9 t-2 to 3), inaequaliter echinulatis et reticulatis, 8–8.75 μ × 10 μ.

TYPE LOCALITY: Rock Springs (Kelly Park), Orange Co., Florida.

HABITAT: In humus under live and water oaks.

DISTRIBUTION: Rock Springs and Longwood, Florida.

This species is characterized by its short stipe, slimy pileus with the agglutinated fibrous marginal area, and the unchanging white acrid latex. Rarely a specimen was found with some spots on the surface of the pileus giving it a subzonate appearance. The fibrous margin and the color separate it from *Lactaria hysgina* Fries, and the unchanging color of the latex on the wounds is an additional characteristic separating it from *Lactaria trivialis* Fries. The spores are also paler than in either of these species.

Lactaria impercepta sp. nov. (FIG. 1, *C*; 4, *F*)

Pileus from broadly convex spreading and slightly umbonate, 5.5 cm. broad; surface dark fawn to snuff brown tone 2, azonate, viscid when wet, glabrous; margin becoming striate on the edge when mature; context staining sulphur yellow when cut, without odor; latex white, slowly becoming sulphur yellow, bitter, then peppery; lamellae pallid, tinted rosy-white tone 4 singly, broad and rounded at the outer end, narrowed toward the inner and a little

decurrent, unequal, sometimes forked at the stipe, close; stipe pale flesh tone 4, 3.5 cm. × 1 cm. at the apex to .8 cm. at the base which is white tomentose, solid: spores fleshy-white tone 1, 8.7–9 μ × 10–11.25 μ, reticulate with small protuberances, apiculate, unsymmetrical.

Pileo e lato convexo expanso, subumbonatoque, 5.5 cm. lato, fuscefulvo (307 to 303 t-2), aut Havanna-brunneo (303), azono, viscido udo, glabro; margine demum striato; carne vulnerata lutescente, inodora; lacte albo, tarde sulphurescente (18), amaro, tum acri; lamellis subincarnatis (8 t-4), latis, exto rotundatis, ad stipitem attenuatis, subdecurrentibus, inequalibus, interdum ad stipitem furcatis, confertis; stipite pallide incarnato (136 t-4), 3.5 × 1 cm., deorsum constricto et albo-tomentoso, solido; sporis albidis (9 t-1) 8.7–9 μ × 10–11.25 μ, tuberculeis-reticulatis, apiculatis inequalibus.

TYPE LOCALITY: Black Hammock opposite the flowing sulphur well, near Oviedo, Florida.

HABITAT: In a hammock of sweet gum, maple and oaks.

DISTRIBUTION: In type locality and in woods three miles from Apopka on the road to Rock Springs, Florida.

This species seems to be rare but it may be because it has been overlooked. It differs from *Lactaria cognoscibilis* in being azonate and in the changing color of the latex and cut flesh and in the larger more reticulate spores. From *Lactaria theiogala* (Bull.) Fries it differs in the more slender form, azonate pileus, less crowded lamellae, more slowly changing latex and absence of odor. In fact in the field it would not suggest any of these species.

Lactaria pseudodeliciosa sp. nov. (FIG. 3, *A;* 4, *G*)

Pileus broadly convex, deeply umbilicate, expanding and infundibuliform, 6 to 8.5 cm. broad; surface nearly white becoming with age over the central portion putty color tone 1 to ochroleucous or maize tone 2 to pale buff, azonate to faintly zonate, very viscid when wet; margin thin, agglutinated fibrous under the lens, easily visible when young; context without odor, turning drab green where wounded; latex neutral orange, scanty, slowly peppery; lamellae honey yellow tone 1 singly except at the base which is orange and gives orange tones to the lamellae in position, unequal, interveined, some anastomosing at the base, adnate to adnate-decurrent; stipe isabelline tone 1–4, somewhat scrobiculate, white tomentose on the lower half, short, solid, 1.2 cm. × 1 cm. to 2.5 cm. × 2 cm., extending to a root-like point on one side: spores maize 2–3, 6.87–7.5 μ × 8.75–9 μ, reticulate and minutely tuberculate, unsymmetrical, apiculus small.

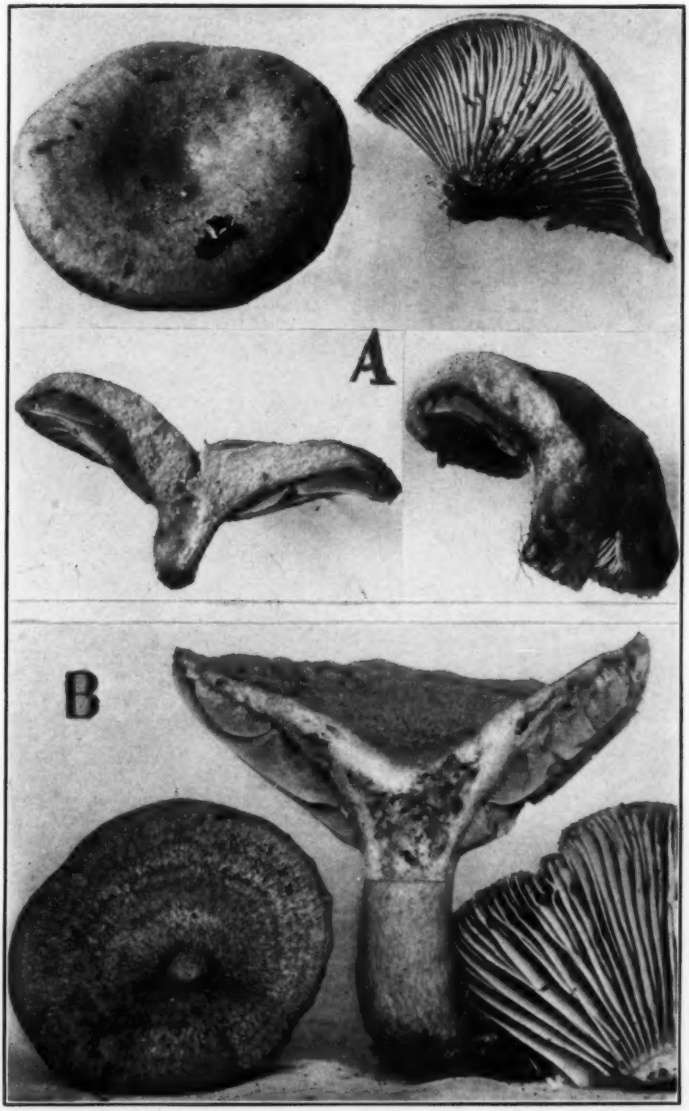


FIG. 3. *A, Lactaria pseudodeliciosa* × 1, often larger; *B, Lactaria paradoxa* × 1.

Pileo late convexo, profunde umbilicato, tum expanso, et infundibuliformi, 6–8.5 cm. lato, subalbo, disco e pallido ochroleuco, azono aut interdum leviter zonato, viscosissimo; margine tenui, minute fibrillis agglutinatis tomentoso; carne inodora, vulnerata virescente; lacte exiguo, aurantiaco, tarde acri; lamellis melleis, basi aurantiacis, inaequalibus, intervenosis, paucis basi cohaerentibus, adnatis aut adnato-decurrentibus; stipite isabellino, leniter scrobiculato, infra albo-tomentoso, curto, solido, 1.2 × 1 cm. ad 2.5 × 2 cm., basi unilatere acuto-constricto pseudo-radicanteque: sporis pallide luteis (36 t 2–3), tuberculis et reticulatis, 6.87–7.5 μ × 8.75–9 μ.

TYPE LOCALITY: Rock Springs, (Kelly Park), Orange Co., Florida.

HABITAT: Under laurel leaved oaks and long leaf pine in a rather open place where the sun could reach during some part of the day.

DISTRIBUTION: Rock Springs, near Oviedo, Longwood and New Smyrna, Florida.

This species seems closely related to *Lactaria deliciosa* (L.) Fries from which it differs in the agglutinated fibrous edge as seen with a lens, the nearly white and usually azonate pileus, the lack of odor, the smaller more finely echinulate spores connected by finer lines, and the small apiculus. In the field the pileus is practically white before it begins to age.

Lactaria paradoxa sp. nov. (FIG. 3, *B*; 4, *H*)

Pileus fleshy, broadly convex becoming centrally depressed and at length infundibuliform, grayish indigo to smalt blue zoned with darker tones, with a silvery sheen over all, fading with age, very viscid when wet, glabrous, 5.2 cm. to 8 cm. broad; margin thin even, showing a tint of the latex when mature; context with a tinge of mahogany-red due to the latex, odorless; latex mahogany-red, a little bitter or astringent, then slowly a little peppery; lamellae putty color to cinnamon singly, in position showing a tint of corinthian red due to the latex, becoming green where injured, unequal, a few forking near the stipe, adnate to decurrent, broad, brittle, easily bleeding; stipe tinged slate violet to smalt blue at the base, equal or tapering downward, and at the base tapering into a root-like extension, 2–3 cm. × 1.2–1.5 cm. at the apex to .8 cm. above the root-like projection; spores pale ecru tone 4 to maize tone 2 in deep mass, broadly elliptical, reticulate with rather broad bands and with small protuberances of unequal sizes, 6.87–7.5 μ × 8–9 μ.

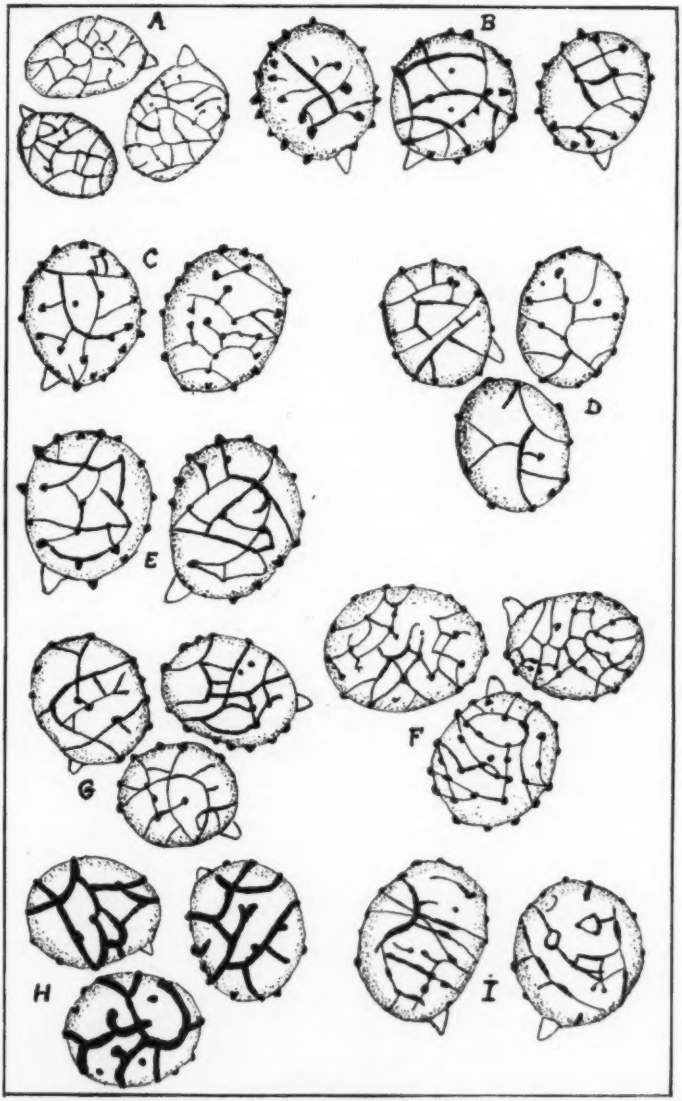


FIG. 4. Spores of *A, Lactaria floridana; B, L. cognoscibilis; C, L. proximella; D, L. torminosa* from specimens collected in Sweden; *E, L. limacina; F, L. impercepta; G, L. pseudodeliciosa; H, L. paradoxa; I, L. subpurpurea.*

Pileo carnoso, late convexo, demum centro depresso et infundibuliformi, caeruleo (232 ad 209), obscurioribus zonis, demum aetate pallidiore, udo viscosissimo, glabro, 5.2 ad 8 cm. lato; margine tenui, laevi; carne lacte colorato, inodoro; lacte fusco-rubro (335), subamaro aut astricto tum tarde acri; lamellis e luteolis (311) cinnamoneis (323) aut lacte rubescentibus, vulneratis virescentibus, inaequalibus, paucis ad stipitem furcatis, adnatis deinde decurrantibus, latis, fragilibus, lacte faciliter plurantibus; stipite supra livido (173) basi caeruleo (209), aequali aut infra constricto et subradicante, 2–3 cm. × 1.2–1.5 cm.; sporis pallide luteis (66 ad 36 t-2), late ellipticis, latis lineis parvis tuberculis late reticulatis 6.87–7.5 μ × 8–9 μ.

TYPE LOCALITY: Fort Christmas Land Development Co. hammock at "Old Faithful," near Fort Christmas, Florida.

HABITAT: In somewhat grassy places under cabbage palmetto and live oaks in a fairly shady spot in rich soil, or in lawns having similar conditions.

DISTRIBUTION: In the type locality and in a lawn at Altamonte Springs, Florida.

When viewed from above in its habitat it closely resembles *Lactaria Indigo* (Schw.) Fries, but upon looking at the under side one is surprised to find it closely resembles *Lactaria subpurpurea* Peck, and the latex is mahogany red instead of blue as one would expect from the color of the upper side of the pileus. The spores differ in size and shape and markings from those of *Lactaria subpurpurea* (FIG. 4, *1*). The spores of the latter species are larger, and the bands are not of uniform width and are more tuberculate.

The spore drawings in figure 4 were made with the aid of a camera lucida, using a 1/12 oil immersion lens and a 15 mm. ocular giving a magnification of about 1500.

THE GENUS MYRIANGIUM IN NORTH AMERICA

JULIAN H. MILLER[1]

This interesting group of plectomycetous fungi occur on scale insects which parasitize various woody plants. They are distributed over the entire world, but are especially common in the southern United States.

The plant body consists of a black pseudoparenchymatous stroma, which later gives rise to a peculiar type of apothecium with an apically delimited fertile region, consisting of asci at different levels embedded in coalesced fungous tissue. The ascospores are liberated by the gelatinization of the surface layer followed by the elongation of the asci, a transverse splitting of the ascal wall, and a forceable expulsion of the spores. There is apparently no conidial stage. The development of the fruiting body has previously been described by the writer (**6**).

The stroma is superficial on the bark and no mycelium has been found in live tissue. Directly under each stroma are several dead scales, penetrated and covered by mycelium. There is a definite relationship between the death of the scale and that of the limb and the *Myriangium*. No live stromata have been discovered on dead branches.

There is apparently some host specificity as regards both the scale and the tree, but in most of the herbarium specimens studied it has been impossible to identify the scale. In the United States the two commonest species are *Myriangium Duriaei* and *Myriangium asterinosporum,* and the former was on every black gum observed by the writer and the latter on nearly every hawthorn. On the other hand these species have also occasionally been found on other hosts, but no cases have been observed where they crossed hosts.

[1] The author wishes to express his appreciation to Dr. F. J. Seaver, The New York Botanical Garden, Dr. D. H. Linder, Harvard University, and Dr. C. W. Dodge, Missouri Botanical Garden, for the loan of specimens.

Records in North American literature of scale insects acting as hosts include the following: *Aspidiotus perniciosus, Chionaspis biclavis, Chionaspis Citri, Chionaspis sylvatica, Diaspis pentagona, Lepidosaphes Becki, Lepidosaphes Gloveri, Lepidosaphes Ulmi, Mytilaspis citricola,* and *Parlatoria Pergandei.* Most of these have been determined on *Citrus* species in Florida or the West Indian Islands, and the fungus has in most cases been wrongly determined as *Myriangium Duriaei.*

Species of *Myriangium* are important in control of scale insects, and have been so cited by South (**14**) and Johnston (**3**) for the West Indies, and by Fawsett (**2**) for Florida.

HISTORICAL REVIEW

Petch (**12**) in 1924 has given a most complete review of the literature of *Myriangium.* Berkeley received specimens from Australia, and on submitting them to Montagne found that he already had collected a similar fungus in the Department of Eastern Pyrenees in France, and had received another one from Algeria. So they (**9**) published the description under joint authorship in 1845. Apparently Berkeley wrote the diagnosis and Montagne (**7**) the next year published a second description and illustrations. Two species were described under the genus, *Myriangium Duriaei* and *Myriangium Montagnei.* The first then becomes the type, and is based on the Montagne collection in the Pyrenees, and the second is from the Australian collection.

In 1849 Berkeley received specimens from M. A. Curtis in South Carolina, which he sent to Montagne (**10**) and the latter then emended the genus and published a third species, *Myriangium Curtisii.*

Both Berkeley and Montagne placed *Myriangium* among the lichens in the family Collemaceae. This was probably due to the greenish tint sometimes found in *Myriangium Duriaei,* and to the disc shaped apothecia and muriform spores, somewhat similar to those of *Collema.* However, there are no internal algal components, although one does occasionally find some algal cells, chiefly *Protococcus,* on the surface of an old stroma.

Nylander in "Synopsis methodica Lichenum" in 1858 erected

a family Myriangiaceae with a tribe Myriangiei, and included the two species, *Myriangium Duriaei* and *Myriangium Curtisii.*

In the United States, Tuckerman in "Genera Lichenum" in 1872 shows that *Myriangium Duriaei* of Europe is equal to *Myriangium Curtisii* of America. Later in 1882 he (**16**) lists *Myriangium Duriaei* (Mont. & Berk.) Tuck., and cites as synonyms *Myriangium Duriaei* Mont. & Berk. and *Myriangium Curtisii* Mont. & Berk. There was no reason for this transfer of a species already in the genus. This inclusion in the lichens by Tuckerman was followed by many North American collectors.

Millardet (**5**) was one of the first to recognize the relationship with the fungi and placed the family Myriangiaceae near the Tuberaceae.

The writer (l.c.) in 1938 summarizes the question of the relationship of *Myriangium* species. The fact that the single ascal locule is formed from ascogenous hyphae clearly indicated a close affinity to the Gymnoascales.

SYSTEMATIC ACCOUNT

MYRIANGIUM Mont. & Berk. London Jour. Bot. **4**: 72. 1845.
Emended Mont. Ann. Sci. Nat. III, **11**: 245. 1849.
Phymatosphaeria Pass. Nuov. Giorn. Bot. Ital. **7**: 138. 1886.
Pyrenotheca Pat. Bull. Soc. Bot. Fr. **33**: 155. 1886.

Stroma superficial, black with lighter colored interior, orbicular to irregular, no free mycelium; apothecia few to many seated on the stroma and consisting of a differentiated fertile layer with asci at different levels above a sterile region; asci globose to subglobose, with 8 spores; ascospores elliptical to oblong, muriform, hyaline to subhyaline.

On *Coccidae* on woody plants.

The genus is limited to the original concept of a stroma with many apothecia parasitizing scales and superficial on the bark. Such plant parasites as *Diplotheca tunae* (Spreng.) Starb., although placed in *Myriangium* by Petrak (**13**) differ sufficiently in lacking the flat basal stroma and in parasitism to warrant the retention of *Diplotheca.* The only other species described from North America, *M. Sabaleos,* by Weedon (**17**) does not belong in the *Myriangium* connection.

The use of the term apothecium for the fruiting body of *Myriangium* is questionable, but seem preferable to such terms as perithecium or cleistothecium, which have been applied to other plectomycete forms as those in *Aspergillus* or *Penicillium*. The asci in *Myriangium* push up through the ascogenous hyphae at maturity, and the fertile region is fully exposed, which is different from the situation in *Aspergillus*. There one finds a sterile crust entirely inclosing the asci and the spores are liberated only after the decomposition of the peridium. The only distinction between the exposed ascal region in *Myriangium* and that of a typical discomycete lies in monostichous asci in the latter and polystichous ones in the former, and that doesn't seem sufficient to warrant coining a new term.

Petch (l.c.) observed some globose spores in several species of *Myriangium*, but the writer has found only oblong-elliptical ones in parts of the same specimens he examined. The spherical shape appears only in cross sections of spores as shown by the figures of the writer (l.c.).

KEY TO NORTH AMERICAN SPECIES OF MYRIANGIUM

Fertile region arising from the inside of a marginate, disc-shaped, stalked apothecium, with concave line separating it from the stroma. 1. *M. Duriaei.*

Fertile region superficial from the first, both upper and lower lines more or less parallel and convex to flat; apothecia emarginate.

Apothecia well differentiated on a discrete stroma, 1–2 mm. high.

Interior of stroma yellow. .....2. *M. asterinosporum.*

Interior of stroma reddish-brown. .....3. *M. tuberculans.*

Apothecia sessile, rounded, or truncate when crowded, scarcely raised above the surface of a flat indefinite stroma. ....4. *M. floridanum.*

1. MYRIANGIUM DURIAEI Mont. & Berk. London Jour. Bot. **4**: 72. 1845.

Myriangium Curtisii Mont. & Berk. Ann. Sci. Nat. III. **11**: 245. 1849.

TYPE: Specimen in Montagne herbarium on *Morus alba*, collected in France in 1830.

Stroma black with pale interior, fleshy to gelatinous when wet, flat to convex, orbicular, plicate-radiate, 1.5–5 mm. in diam.; few to many apothecia arising as erect processes with depressed center, with fertile region in the upper part, at first closed, but fully ex-

posed at maturity, disc-shaped, stalked, .5–1.5 mm. in diam. and .5–1 mm. high, with distinct margin, surface plane or convex becoming concave with drying or discharge of spores; asci globose to semiglobose, 35–50 μ in diam., with thick hyaline wall, 8-spores; ascospores oblong-elliptical, muriform, constricted at middle septum, 7–9 transverse septa and 1–3 longitudinal divisions, subhyaline, 25–36 $\times$ 12–14 μ.

In North America it is most common on *Nyssa sylvatica* and *Nyssa biflora,* but also occurs on *Heteromeles arbutifolia, Liquidambar Styraciflua, Magnolia virginiana,* and *Quercus nigra.* In Europe the collections are chiefly on *Fraxinus excelsior,* and *Morus alba* and in Africa on *Laurus nobilis.* The distribution of the species is apparently world wide.

ILLUSTRATIONS: Petch (l.c.) *pl. II, f. 1–4,* sub *M. Duriaei,* and *f. 6* sub *M. Curtisii,* and *pl. III, f. 1–4.* The *f. 4* is named *M. Curtisii,* but is a typically expanded *M. Duriaei.* Miller (l.c.) *f. 4, 1–5.*

The apothecia of *Myriangium Duriaei* in most herbarium specimens are either immature or else old and disintegrated. This is due to the fact that in the absence of a prolonged wet period they fail to develop and may remain in an arrested condition of rudimentary tubercules for many months, and so the periods in which they can be collected in the mature state is very brief. Petch (l.c.) describes these initials as well as open apothecia without drawing the conclusion that the former are only immature stages of the latter.

The North American form on *Nyssa* is fully equal to the European specimens cited below. The Italian specimen *n. 27* is old, but has typical concave cups with definite borders. The English specimen from Currey on *Fraxinus* is the best developed one of the foreign collections in the New York Botanical Garden herbarium.

Petch (l.c.) cites the New Jersey specimen *n. 143* and the Ravenel collection *n. 332,* all on *Nyssa,* as typical of this species, which is in agreement with this paper.

MATERIAL EXAMINED

United States

Curtis Collections, Harvard Univ., *M. Duriaei* Mont. & Berk. on *Nyssa,* South Carolina. *M. Duriaei* on *Nyssa,* S. C., ex herb. C. D. Faxon. *M. Duriaei* on *Nyssa,* S. C., det. R. Thaxter. *M. Curtisii* Mont. & Berk. E. Tuckerman herb., ex Michener, Chester, Pa., 1852. 2 specimens. (Probably co-type of *M. Curtisii.*)

Ravenel Collections, *M. Curtisii* Mont. & Berk., South Carolina, Apr. 1859, ex Tuckerman herb., 2 specimens. *M. Curtisii,* S. C., ex Gray herb. *M. Curtisii,* on *Nyssa,* Santee Canal, S. C., 1849, Harvard herb. *M. Curtisii,* on *Nyssa,* S. C., Mo. Bot. Gard. herb. no. *34895. M. Curtisii,* on *Nyssa,* Aiken, S. C., Rav. Fungi Am. *332,* 2 specimens. N. Y. Bot. Gard.

Ellis Herb., *M. Duriaei* Mont. & Berk., on *Nyssa,* Newfield, N. J., May 25, 1900. Harvard herb. *M. Duriaei,* on *Nyssa,* Newfield, N. J., 2 specimens, n. *23,* N. Y. Bot. Gard. herb. *143. M. Duriaei,* on *Nyssa multiflora,* Acto, N. J., H. A. Green, 3 specimens in N. Y. Bot. Gard. herb. and *2* in Mo. Bot. Gard. herb. *270. M. Duriaei,* Charleston, S. C., Apr. 1895, H. A. Green. *M. Duriaei,* Chester, S. C., H. A. Green, N. Y. Bot. Gard. herb. *M. Duriaei,* on *Nyssa,* Takoma Park, Md., Mar. 1900, ex herb. T. A. Williams. *1060. M. Curtisii,* on *Nyssa multiflora,* Wilmington, Del., Oct. 1889, ex herb. A. Commons. N. Y. Bot. Gard. herb.

Calkins Collections, *35. M. Duriaei,* Florida, June 17, 1903, N. Y. Bot. Gard. herb. *66. M. Duriaei,* Lookout Mt., Tenn., N. A. Lichens. *74. M. Duriaei,* Cook Co., Ill., N. A. Lichens, Harvard herb. *136. M. Duriaei,* Lookout Mt., Tenn., N. A. Lichens, Mo. Bot. Gard. herb.

Earle, F. S. *2241. Dothiora asterinospora* Ellis & Ev., on *Nyssa,* Auburn, Ala., Jan. 16, 1897, N. Y. Bot. Gard. herb.

Faxon, C. D. *M. Duriaei,* Kaiser, W. Va., Dec. 12, 1880, Harvard herb.

Hasse, H. E. *270. M. Duriaei* (Mont. & Berk.) Tuck., on *Heteromeles arbutifolia,* Santa Monica, Calif., 1898. N. Y. Bot. Gard. herb., also 2 specimens in Harvard herb.

Linder, D. H. & R. F. Smart. *M. Duriaei,* on *Nyssa sylvatica,* Richmond, Va. Harvard herb.

Rapp, S. *14. Myriangium* sp., Sanford, Fla., on *Magnolia virginiana,* Dec. 25, 1907. *174. M. Duriaei,* Sanford, Fla., 1910. Harvard herb.

Miles, L. E. *777. M. Duriaei,* on *Liquidambar Styraciflua,* Auburn, Ala., Apr. 1923. Harvard herb.

Thaxter, R. *4108. M. Duriaei,* West Palm Beach, Fla., Jan. 2, 1897. det. Petch. *4109* is a duplicate. Harvard herb.

Weir, J. R. & W. W. Diehl. *M. Curtisii,* on *Nyssa sylvatica,* Fosteria, Va., Mar. 4, 1932. Harvard herb.

In addition to the above the writer has collections from Georgia on *Nyssa biflora, Nyssa sylvatica, Liquidambar Styraciflua,* and *Quercus nigra.*

South America

Thaxter, R. *4106. M. Duriaei.* Corral, Chile, Dec. 1905. det. Petch. *4107* is a duplicate. Harvard herb.

Europe

Roumeguere, C. *9. M. Duriaei,* Lichenes Gallica exsicc.

Desmazieres, J. B. & H. J. *M. Duriaei,* Plantes Crypt. de France.

Solis, A. L. *M. Duriaei,* Urville, Hague pres Cherbourg, 1857.

Richard, O. J. *M. Duriaei,* Lichens of Cherbier, Mar. 1879. On *Fraxinus excelsior,* Vezuley. 2 specimens.

Currey, W. *M. Duriaei,* on *Fraxinus excelsior,* Apr. 1876.

Massalongo, A. B. *27. M. Duriaei,* Lichens Italici exsicc.

The above are all in the New York Botanical Garden herbarium.

Africa

Maire, R. *45. M. Duriaei,* on *Laurus nobilis, Aonidia Lauri* Bouche, Feb. 1912, Mycotheca Boreali-Africana. N. Y. Bot. Gard. herb.

2. **Myriangium asterinosporum** (Ellis & Ev.) comb. nov.

Cenangium asterinosporum Ellis & Ev. Bull. Torrey Club **10**: 76. 1883.

Dothiora asterinospora Sacc. Syll. Fung. **8**: 766. 1889.

TYPE: Ellis herb. no. *1279,* on *Vaccinium corymbosum.*

Stroma black, flat at first, later hemispheric, orbicular, radiate-plicate on the margin, yellow inside, superficial, 2–5 mm. in diam., and 1–2 mm. high; apothecia many on stroma, usually leaving no free margin, sessile, orbicular to angular when crowded, .1–.8 mm. in diam., chiefly .4 mm., discrete, slightly elevated, black, with wide base, convex to flat; fertile region occupying upper portion of apothecium, exposed from the first; asci semiglobose to globose, 35–52 μ in diam.; ascospores 8, oblong-elliptic, 25–34 $\times$ 9–14 μ, muriform, constricted at middle septum, 7–9 septate and with 1–3 longitudinal divisions, hyaline to light yellow.

HOSTS: Chiefly on *Crataegus* spp., but also found on *Acer, Amelanchier, Cyrilla racemiflora, Ilex decidua, Ilex lucida, Ilex verticillata, Malus angustifolia, Malus pumila,* and *Vaccinium corymbosum.*

DISTRIBUTION: Apparently limited to the United States and Canada, especially the eastern parts.

ILLUSTRATIONS: Miller (l.c.) *f. 4, 6, 7.*

This species was designated *M. Curtisii* by the writer (l.c.) in 1838, and also by Petch (l.c.) in part, but recent investigations lead to the conclusion that Montagne had both of the common American species before him and combined the two in his description of *M. Curtisii.*

The type of *M. Curtisii* is a Curtis specimen collected in South Carolina and sent to Berkeley. According to Petch (l.c.) Berkeley forwarded this one on to Montagne, and the latter described it under joint authorship. Also, Petch says there is no specimen in the Berkeley herbarium labelled *M. Curtisii* by Berkeley, and that in the Montagne herbarium there is one labelled *M. Curtisii* Mont. & Berk. in Broome's handwriting with " Mont. Ann. Sci. B. t. 12, 1849," added by Montagne, which is *M. Duriaei.* Then there is another specimen marked by Montagne " *Myr. Curtisii* B. & M. Car. Inf. Amer. Boreal," which Petch says has the large apothecia of *M. Curtisii* and is probably the type. However, this cannot be determined with any degree of certainty as Montagne did not cite either a number or the host in his description.

In the Farlow Herbarium at Harvard there is a specimen from Curtis labelled *M. Curtisii* from the Tuckerman herbarium, which should be the cotype. This is on *Nyssa* and has large apothecia, and is distinctly *M. Duriaei.* Then Montagne in Sylloge Plant.

Cryptogamarum p. 381 cites both Curtis and Ravenel as sources of *M. Curtisii,* and the Ravenel specimens under that name in the Harvard herbarium, the New York Botanical Garden herbarium, and in the Missouri Botanical Garden herbarium are all on *Nyssa* and are *M. Duriaei.*

The Montagne description of *M. Curtisii* is also confusing. In regard to the apothecia he (**10**) says, ". . . sesselia, haud adnata, elevato-marginata, scutelliformia, concaviuscula . . ., disco subconcolori . . .," and earlier under the *M. Duriaei* description there is "Apothecia tuberculiformia primo clausa, tanden aperta plana immarginata." The common form on *Crataegus,* or the Thaxter specimen on *Amelanchier,* which Petch determined as *M. Curtisii,* has no elevated margin, is never concave, and disc is not concolorous but black. Therefore, as both descriptions fit *M. Duriaei,* and the Curtis and Ravenel specimens named *M. Curtisii* are in reality *M. Duriaei,* the Ellis name *asterinosporum* becomes the first to be applied to this species.

Ellis and Everhart (**1**) recognized only one species in North America, *M. Duriaei,* and placed *Cenangium asterinosporum* and *M. Curtisii* in synonymy.

The spore measurements given by Ellis and Everhart for *M. asterinosporum* are 15–20 × 6–8 μ with 3 septa, and Petch says the spores are 16–19 × 7 μ, with some spherical ones 18 μ in diam. These are probably measurements of immature spores as older parts of the Ellis type have spores as given above by the writer.

This species is included in part by Petch (l.c.) under the name *M. Curtisii.* He separates the two common species as follows:

"Stroma at first green internally:
ascigerous region cup-shaped
1. *Myr. Duriaei*

Stroma yellow-brown internally:
asci in a zone parallel to the surface
2. *Myr. Curtisii*"

The above key contains the essential elements separating the two species, but Petch gives as an illustration of *M. Curtisii* a photomicrograph of a section through a typically expanded form of *M. Duriaei.* Also, he cites under *M. Duriaei* the Ellis specimen, *Cenangium asterinosporum,* which is exactly the same as the one on *Amelanchier* at Harvard that he determined as *M. Curtisii.*

MATERIAL EXAMINED

Anderson, F. W. *M. Curtisii* Mont. & Berk., on *Acer,* Newfield, N. J., May 1890. N. Y. Bot. Gard. herb.

Bartholomew, E. *1101. M. Duriaei,* on *Crataegus,* Iowa City, Ia., May 1905, U. S. D. A. Myc. Coll. Mo. Bot. Gard. herb. and Harvard herb. [Collected by F. J. Seaver]

Burke, R. P. *7. Myriangium sp.,* Fungi of Montgomery Co., Ala., N. Y. Bot. Gard. herb.

Dearness, J. *1141. M. Duriaei,* on *Crataegus,* London, Can., Nov. 1903. Ellis & Ev. Fungi Columb. N. Y. Bot. Gard. herb.

Earle, C. F. *Dothiora asterinospora* Ellis, on *Cyrilla racemiflora,* Auburn, Ala., Jan. 1891. N. Y. Bot. Gard. herb.

Ellis, J. B. *1081. Cenangium asterinosporum* Ellis, on *Vaccinium* corymbosum, Newfield, N. J., Dec. 1882; no. *1279* collected Apr. 1883; an unnumbered one, May 23, 1883. N. Y. Bot. Gard. herb. No. *1279* also in Harvard herb.

Fitzpatrick, H. M. *14763. M. Duriaei,* on *Crataegus,* McLean, N. Y., July 1925. Harvard herb.

Greene, H. A. *M. Duriaei* (Mont. & Berk.) Tuck., Tryon, N. C., 1899, det. Merrill. Harvard herb.

Honey, E. E. & Jenkins, A. E. *69635. M. Duriaei,* on *Crataegus,* McLean, N. Y., 1927. N. Y. Bot. Gard. herb.

Jackson, H. S. *6075. M. Duriaei,* on *Crataegus,* N. of Erindale, Ont., Oct. 1933. Harvard herb.

Martin, G. W. *M. Duriaei,* on *Crataegus,* North Liberty, Ia., Apr. 7, 1934. N. Y. Bot. Gard. herb.

Norton, J. B. S. *2503. M. Duriaei,* on *Crataegus,* Webster, Md., Oct. 1889. Mo. Bot. Gard. herb.

Overholts, L. O. *58213. M. Duriaei,* on *Crataegus,* Rock Springs, Pa., May 1921. Mo. Bot. Gard. herb.

Piquet, A. P. D. *M. Curtisii,* Sharon, Mass., det. Thaxter. Harvard herb.

Sheldon, J. L. *2874. M. Duriaei,* on *Crataegus Crus-galli,* Marillo, W. Va., May 20, 1907. Harvard herb.

Thaxter, R. *623. M. Curtisii,* on *Amelanchier,* Tyler City, Conn., Oct. 1888, det. Petch. N. Y. Bot. Gard. herb. Duplicates, nos. *623, 4105, 3864,* Harvard herb. *776. M.* (*Duriaei*) *Curtisii,*

on *Cocci* on *Amelanchier,* Harvard herb. *Myriangium sp.,* on *Ilex verticillata,* New Haven, Conn., 1888, Harvard herb.

The writer has Georgia collections on *Crataegus Collina, C. Crus-galli, C. Michauxii, C. spathulata,* and *C. uniflora;* and one on *C. punctata* from Ithaca, N. Y. Other Georgia collections are on *Ilex decidua, Malus angustifolia* and *Malus pumila.*

3. MYRIANGIUM TUBERCULANS Miles, Mycologia **14**: 80. 1922.

TYPE: Specimen on *Carya Pecan,* in herb. L. E. Miles, Agr. Coll. Miss.

Stroma at first flat, later hemispheric, black, light pinkish-brown inside, orbicular, discrete, radiate-plicate on margin, 1–4 mm. in diam. and 1–2 mm. high; apothecia orbicular to irregular, flattened to convex, .1–.4 mm. high, usually not distinct, but part of a continuous branching system of convolutions occupying the entire stroma, with peripheral fertile regions covering all exposed surfaces; asci semiglobose, 35–55 μ in diameter; 8 spores, oblong-elliptic, with obtuse ends, 7-septate, with 1–2 longitudinal divisions, subhyaline, 25–32 $\times$ 10–14 μ.

On scale insects on *Carya Pecan, Carya alba,* and *Carya sp.* in southern United States.

ILLUSTRATIONS: Miles (l.c.) *pl. 14, f. 1–4.*

The apothecia are not as distinct as in *M. asterinosporum,* but in all other characters there is a marked similarity. The fertile region is convex with parallel lines in sections, and exposed from the first as in the other species. The chief difference lies in the pinkish color of the interior of the stroma in contrast to the light yellow of *M. asterinosporum.* Also the stromal texture remains firm, and does not crumble with age as in the latter.

Petch (l.c.) places this form under *M. Curtisii* (*M. asterinosporum* of this paper), but the writer thinks the difference in stromal color is sufficient to maintain a separate species.

MATERIAL EXAMINED

Miles, L. E. *115. M. tuberculans,* on *Carya Pecan,* Pascagonla, Miss., May 8, 1921. *M. tuberculans,* on *Carya Pecan,* Ocean Springs, Miss. N. Y. Bot. Gard. herb. *785. Myriangium sp.* on pecan, Selima, Ala., Mar. 10, 1923. Harvard herb.

Demaree, J. B. *1544. M. Duriaei,* on *Carya Pecan,* Putney, Ga., Mar. 15, 1922, Harvard herb. and Mo. Bot. Gard. herb.

In the writer's herbarium this species is on *Carya Pecan, Carya alba,* and *Carya sp.* from Georgia.

4. MYRIANGIUM FLORIDANUM Höhnel, Sitz-ber. Akad. Wiss. Wien. **118**: 354. 1909.

TYPE: Specimen on *Citrus Aurantium,* Umatilla, Fla., Rehm herbarium, ex Ellis.

Stroma very thin, flat, indefinitely effused, following the contours of the bark, externally black and light straw colored inside; apothecia isolated or crowded, sessile, elevations pulvinate, hemispheric to truncate, .1–.3 mm. high and .3–.6 mm. in diameter, surface black pulverulent, with no free margin; fertile region convex with asci semiglobose to globose, 35–53 μ in diam.; ascospores 8, oblong-elliptic, with 7–9 septa and 1–2 longitudinal divisions, subhyaline, 27.6–32 $\times$ 9.2–12 μ.

On various scale insects on *Citrus Aurantium, Citrus nobilis* var. *Unshiu, Citrus Limonia,* and on *Gleditsia triacanthos.* Found in southern United States, the West Indies, and Africa.

The specimens on *Citrus* species studies by the writer are similar in possession of imperfectly developed emarginate apothecia, completely sessile on the flat basal stroma, with the fertile region not sharply defined. This is in distinct contrast to the cup-shaped apothecia of *M. Duriaei,* or the apothecia of the other species with their raised sterile stroma under each fertile region.

M. floridanum is the most primitive species and is not far removed from some of the more highly developed forms in *Elsinoe.*

The form on *Gleditsia* has been found only in the Mississippi valley states. It does not differ sufficiently from the specimens on *Citrus* to warrant the creation of a separate species. These minor variations consist in a slightly thicker stroma and more indefinite apothecial elevations.

MATERIAL EXAMINED

United States

Dozier, H. L. *M. Duriaei,* on *Citrus nobilis* var. *Unshiu,* Mobile, Ala., Apr. 7, 1923, det. W. W. Diehl. *809. M. Duriaei,* on purple scale on Satsuma orange, Loxley, Ala., Apr. 10, 1923. Harvard herb.

Ellis herb. *M. Duriaei,* on orange, Umatilla, Fla., C. W. Hopkins, with drawings by E. A. Southworth. N. Y. Bot. Gard. herb. *778. M. Duriaei,* on orange twigs, Florida, det. Thaxter. Harvard herb. ex Ellis.

Savage, F. *1547. M. Duriaei,* with *Mytilaspis Citri* on *Citrus trifoliata,* Eustis, Fla., Jan. 1902. U. S. D. A. Myc. Coll. Mo. Bot. Gard. herb., Harvard herb. and N. Y. Bot. Gard. herb.

West Indies

Britton, N. L. *13291. M. Duriaei* ?, on *Pseudocarpidium Wrightii,* at Palm Barren, Santa Clara, Cuba, Apr. 8, 9, 1912. N. Y. Bot. Gard. herb.

Greene, J. *1546. M. Duriaei,* with *Mytilaspis citricola* & *Chionaspis citri,* on lemon twig, Oriente, Cuba, Nov. 29, 1909. U. S. D. A. Myc. Coll. Harvard herb., Mo. Bot. Gard. herb., and N. Y. Bot. Gard. herb.

Seaver, F. J. & Chardon, C. E. *343. M. Duriaei,* on oyster shell scale, Puerto Rico. *1660. M. Duriaei,* on scale insect, Rio Portugues n. of Ponce, Explor. of P. R. n. *395. 2081 M. Duriaei,* Jan. 24, 1923, Explor. of P. R. n. *1071. 1983. M. Duriaei,* Explor. of P. R. n. *718,* on Bromeliad. N. Y. Bot. Gard. herb.

Seaver, F. J. *3435. M. Duriaei,* on insect, Plants of Trinidad, Br. W. I., Apr. 3, 5, 1921. N. Y. Bot. Gard. herb.

Africa

Humber, T. *M. Duriaei,* on orange, Coomassie, Gold Coast, Africa, 1917. Harvard herb.

UNITED STATES

On Gleditsia

Ellis Herb. *4067. M. Duriaei* Mont. & Berk., on *Gleditsia triacanthos,* Fountain Bluff, Ill., May, 1894. Rab.-Wint.-Pazsch. Fungi Europaei. N. Y. Bot. Gard. herb.

Langlois, A. B. *94. M. Curtisii* Mont. & Berk., on *Gleditsia,* Point a la Hache, La. Dec. 8, 1885. Flora Ludoviciana. *M. Cur-*

tisii, on *Gleditsia*, St. Martinsville, La., Apr. 15, 1895. N. Y. Bot. Gard. herb.

Miles, L. E. *Myriangium* sp., on *Gleditsia triacanthos*, Miss. A. & M. College, Apr. 4, 1922. N. Y. Bot. Gard. herb.

Tracy, S. M. *182. M. Curtisii*, on *Gleditsia triacanthos*, Starkville, Miss., Mar. 1, 1889. Mo. Bot. Gard. herb. and N. Y. Bot. Gard. herb.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF GEORGIA,
ATHENS, GEORGIA

LITERATURE CITED

1. **Ellis, J. B. & Everhart, B. M.** The North American Pyrenomycetes. 793 pp. Newfield, N. J. 1892.
2. **Fawsett, H. S.** Citrus diseases and their control. McGraw-Hill Pub. Co. 2nd. Ed. 656 pp. 1936.
3. **Johnston, J. R.** Entomogenous fungi of Puerto Rico. P. R. Agr. Exp. Sta. Bull. **10**: 1–33. 1915.
4. **Miles, L. E.** A new species of *Myriangium* on pecan. Mycologia **14**: 77–80. 1922.
5. **Millardet, M. A.** Des genres, *Atichia*, F. W., *Myriangium* Mont. and Berk., *Naetrocymbe* KBR., Memoires pour Servir à l'histoire des Collémacées. Mem. Soc. Sci. Nat. Strasbourg **6(2)**: 1–22. 1870. *Illus.*
6. **Miller, J. H.** Studies in the development of two *Myriangium* species and the systematic position of the order Myriangiales. Mycologia **30**: 158–181. 1938.
7. **Montagne, C. L.** Exploration Scientifique d'Algerie **1**: 213. *pl. 19, f. 2.* 1846.
8. ——. Syll. Plant. Crypt. pp. 380–1. 1856.
9. —— **& Berkeley, M. J.** London Jour. Bot. **4**: 72. 1845.
10. —— & ——. Ann. Sci. Nat. III **11**: 235–246. 1849.
11. **Nylander, W.** Synopsis methodica lichenum, omnium hucusque cognitorum praemissa introductione lingua Gallica tractata. Tome 1, 430 pp. *illus.* Paris. 1858–60.
12. **Petch, T.** Studies in entomogenous fungi. V. *Myriangium*. Trans. Brit. Myc. Soc. **10**: 45–80. 1924.
13. **Petrak, F.** Mykologische Notizen. 632. Über *Sphaeria tunae* Spreng. und die Gattung *Diplotheca* Starb. Ann. Myc. **27**: 357–359. 1929.
14. **South, F. W.** The control of scale insects in the British West Indies by means of fungoid parasites. W. I. Bull. **11**: 1–30. 1910.
15. **Tuckerman, E.** Genera Lichenum. p. 141. 1872.
16. ——. Synopsis of North American Lichens. p. 261. 1882.
17. **Weedon, Amy B.** Some Florida fungi. Mycologia **18**: 218–223. 1926.

ANGULAR LEAF SPOT OF PITTOSPORUM

A. G. PLAKIDAS

(WITH 4 FIGURES)

In June, 1938, Mr. L. A. Hetrick, then with the State Department of Agriculture, sent from New Orleans specimens of *Pittosporum* (*P. tobira*) leaves showing very striking yellowish-brown angular spots (FIG. 1, *A*). Examination of the specimens showed fungus mycelium within the spotted leaf tissue and sporophores (apparently those of *Cercospora*) protruding in fascicles from the stomata. A few spores were also present. No published account or description of the disease was found. Later, the disease was found on *Pittosporum* shrubs on the University campus, and in gardens in Baton Rouge, New Orleans, Thibodaux, and other places in southern Louisiana and along the Mississippi coast.[1]

DESCRIPTION

The spots are characteristically angular (FIG. 1, *A*) and chlorotic, but usually not necrotic. Completely necrotic spots occur only rarely. The invaded tissue remains alive for a long time. For example, leaves infected by artificial inoculation in May, 1939, still showed chlorotic but no necrotic spots on January 8, 1940, about seven months after the appearance of the spots. It is true that if the tissue of even relatively young spots is sectioned and examined with the microscope, groups of necrotic spongy parenchyma and palisade cells are found, chiefly in the vicinity of fungus mycelium, but, on the whole, the invaded tissue remains alive. This is a very interesting feature of this particular disease, for fungi of this genus (*Cercospora*) usually behave as aggressive parasites, killing the host tissue and causing necrotic spots shortly after infection has taken place. In the early stages, the spots are pale green in

[1] Recently Dr. G. F. Weber informed the writer that the disease described in this paper is present in Florida. Press Bulletin 520, with the title "Leaf Spot of Pittosporum," by E. West, released by the Florida Agr. Exp. Sta. in August, 1938, came to the writer's attention recently.

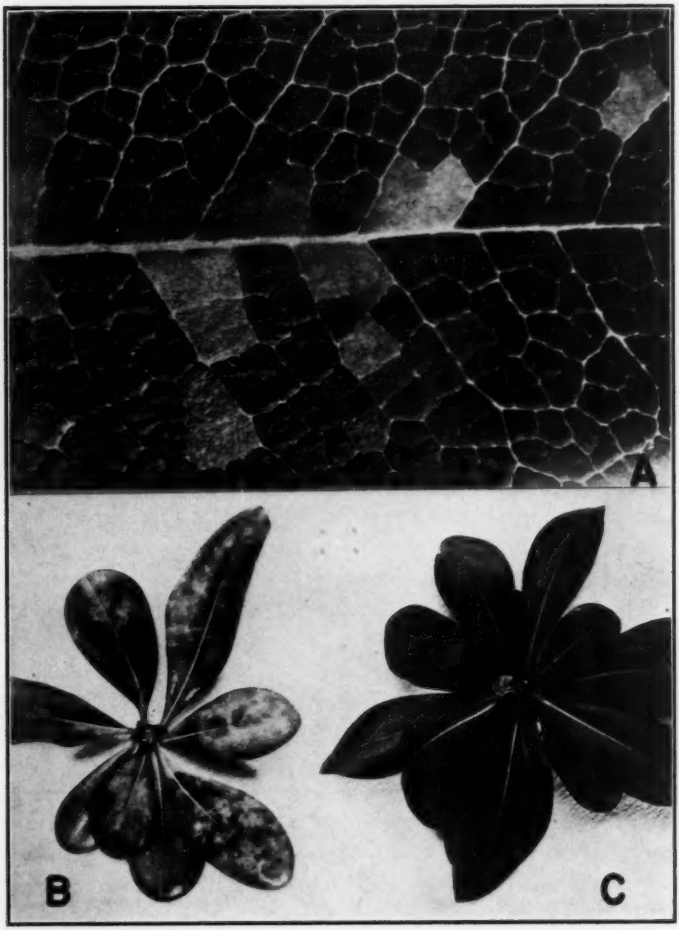


FIG. 1. Angular leaf spot of *Pittosporum tobira*. *A*, portion of a leaf showing typical angular chlorotic spots, × 5; *B*, shoot showing very severe symptoms, with more than one-half of the leaf area covered with chlorotic spots; *C*, healthy shoot for comparison. *B* and *C* approx. ½ nat. size.

color and hardly discernible unless viewed through transmitted light. Later, the spots become distinctly chlorotic, and still later yellowish-brown on the upper and olive brown on the under surface. Solitary spots may vary from 1 to 5 mm. in size. Coalescing spots are much larger, often covering more than one-half the area of the leaf (FIG. 1, *B*), but even the large coalescing spots have angular margins. The angularity of the spots is due to the fact that the spread of the fungus through the tissue is checked, temporarily at least, by the veinlets, so that the margins of the spots follow the outline of the veinlets. It is only rarely that a spot may show a diffuse margin.

Mycelium occurs in the affected tissue between both the spongy parenchyma and the palisade cells. It is especially abundant directly under the lower epidermis where it forms a rather dense growth in the sub-stomatal air spaces (FIG. 2), from which the sporophores arise and protrude through the stomata (FIG. 3, *A*).

The disease occurs on both the green and the variegated varieties of *Pittosporum*. In general, it is not a serious disease, for, although some spotting may be found on practically every *Pittosporum* shrub in certain areas, it is relatively seldom that the infection is severe enough to cause serious damage. However, many individual shrubs have been noted that were so heavily spotted as to present a chlorotic, sickly, unsightly appearance (FIG. 1, *B*). It often happens that a severely-diseased shrub may be growing next to, or between, other shrubs that show only an occasional spot, and the disease does not seem to spread to them, although the cultural and environmenal conditions appear identical for all. No satisfactory explanation can be offered at present for this apparent difference in susceptibility. As far as it could be determined, all the *Pittosporums* growing in the region under observation belong to the same species (*P. tobira*), and, since they are propagated vegetatively, all plants should be the same, unless clonal mutations in respect to susceptibility occur. Furthermore, when old plants which showed only occasional spots were inoculated artificially, severe infection was obtained, showing that these plants do not possess any inherent resistance. Since the infected leaves do not fall off, the disease persists throughout the winter and summer,

although infection appears to take place only in the warm season. Inoculations made outdoors in November did not cause infection.

PATHOGENICITY

The disease was produced in its typical form by inoculating outdoors attached shoots of *Pittosporum* shrubs. Inoculations were made by the following methods: (1) spraying the leaves by means of a hand sprayer with a spore suspension in water, the spores being taken from naturally infected leaves; (2) transferring dry spores by means of a brush from naturally infected leaves to the undersurfaces of healthy leaves; (3) placing infected leaves next to healthy ones in such a way that their under surfaces were in contact; (4) spraying with a mycelial suspension in water of a pure culture of the fungus. Non-inoculated checks were also pro-

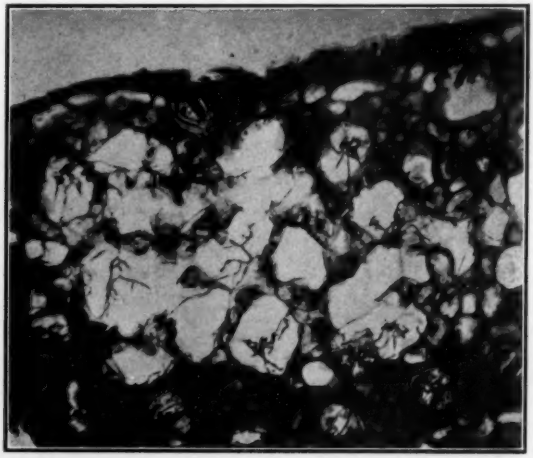


FIG. 2. Surface section of leaf showing profuse growth of mycelium beneath the lower epidermis with denser growth in the substomatal cavities. × 190.

vided. Some of the inoculated shoots in each series were placed under bell jars and some were left uncovered, but apparently covering the inoculated shoots with bell jars was unnecessary as the amount of infection on the covered shoots was not greater than

on those left exposed. The inoculations were made between May and September when the atmospheric humidity was very high.

Results: Heavy infection was obtained in every case with methods 1 to 3, that is, when the inoculum consisted of spores from infected leaves. With pure culture inoculum, infection was very light (12 spots in one experiment and 7 in another). This was probably due to the fact that the cultures used as inoculum were not sporulating. Spots appeared on the inoculated leaves between 3 and 4 weeks after inoculation.

THE FUNGUS

The mycelium occurs both internally and externally. The internal mycelium occurs between both the palisade and the spongy parenchyma cells, but most profusely under the lower epidermis and especially in the sub-stomatal cavities where it forms a rather dense growth of interwoven hyphae. The hyphae in the sub-stomatal cavities which give rise to the sporophores do not become thickened, sclerotial, or tuberculate. The external mycelium arises either from the sub-stomatal hyphae, or as branches of the sporophores. In the young spots, the external mycelium is inconspicuous. In the older spots it forms, together with the sporophores and the entangled spores, a felty overgrowth which appears olive-brown in color, although the individual hyphae are hyaline.

The sporophores arise for the most part from the sub-stomatal hyphae of the internal mycelium, emerging in fascicles through the stomata of the lower epidermis (FIG. 3, *A*), though some may arise as branches of the external mycelium. They were never seen breaking through the epidermis. The young sporophores are practically hyaline, becoming only slightly dusky (never decidedly brown) with age. They are septate, alternately branched, procumbent, 3.9–5.8 μ (ave. 4.9 μ) in diameter, and vary considerably in length (FIG. 4). It is difficult to determine accurately the length of the sporophores as some of the branches often elongate forming vegetative hyphae, and it is not always easy to tell where a sporophore ends and the vegetative mycelium begins. Fifty sporophores were measured and these varied in length from 22.0 to 65.0 μ, averaging 35.7 μ.

The conidia (FIG. 3, *B*) are long, slender, hyaline, septate, rarely constricted at the septa, cylindric to narrowly obclavate, straight or curved. They are mostly pointed at the base, and the points of attachment may be rounded or narrowly truncate. The length and degree of septation of the conidia vary with age (and probably with environmental conditions). One hundred spores from rela-

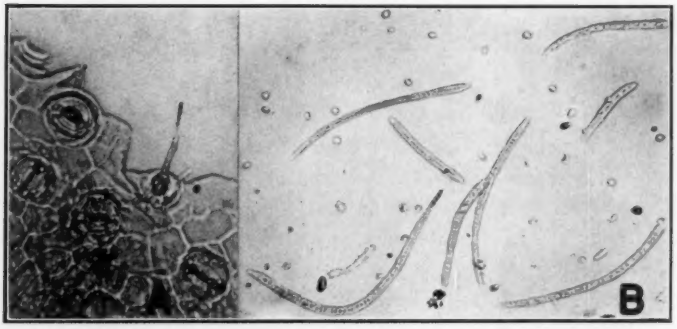


FIG. 3. *A*, free-hand section of lower epidermis showing conidiophores protruding through the stomata, × 230; *B*, conidia, × 350.

tively young spots, measured in May 1939, varied in size from 34–109 × 3.0–4.4 μ, ave. 72.0 × 3.6 μ, and in septa from 3–12, ave. 5.8 per spore. A second lot of 100 spores from older spots varied in size from 54–143 × 3.4–4.4, ave. 84.4 × 3.7 μ, and in septa from 4–13, ave. 7.5 per spore.

The fungus was obtained in culture by tissue transfers and by isolating single conidia. It grows readily, although slowly, on a variety of media, producing olivaceous-gray to dark-gray colonies. Like many other members of this genus, it sporulates rather sparingly in culture. Some of the isolates sporulated more readily than others, but even the recalcitrant isolates were induced to sporulate by macerating the mycelium in sterile water and smearing the suspension on beanpod agar in plates. By this method, conidia were produced fairly abundantly.

TECHNICAL DESCRIPTION

Cercospora Pittospori sp. nov.

Spots angular, vein-limited, chlorotic, rarely necrotic, pale green to yellowish-brown above, light yellow to olive-brown below, solitary spots 1–5 mm., confluent spots much larger often covering more than one-half the area of the leaf. Mycelium internal and external, hyaline, regular to irregular, 2.0–4.4 μ; external hyphae somewhat larger than the internal. Conidiophores hypophyllous, arising from the more or less loose sub-stomatal hyphae of the internal mycelium and emerging in fascicles through the stomata, or scattered on the external mycelium, procumbent, septate, hyaline to

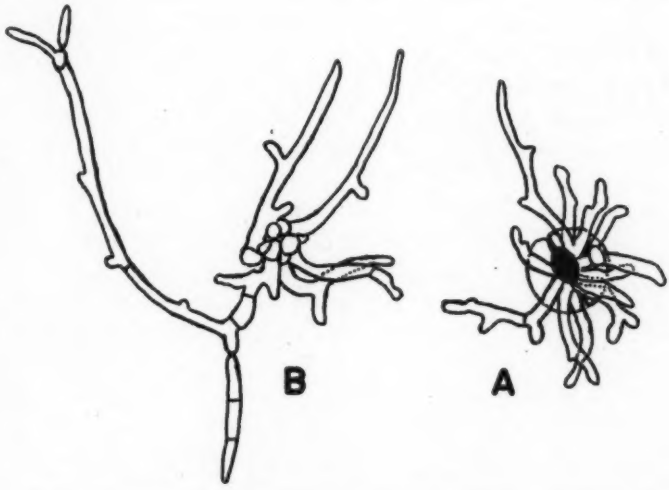


FIG. 4. Camera lucida line drawings of conidiophores of *Cercospora Pittospori*, × 578. *A*, relatively young conidiophores; *B*, older conidiophores considerably elongated.

slightly dusky (never decidedly brown), 22–65 × 3.9–5.8 μ, alternately branched, some of the branches often elongating to form external mycelium. Conidia cylindrical to narrowly obclavate, straight or curved, narrowing at the base with their point of attachment rounded or narrowly truncate, hyaline, 34–143 × 3–4.4 μ, 3–13 septate, occasionally constricted at the septa.

[2] Maculis angularibus, veno-limitatis, chloroticis, raro necroticis, supra e viridi pallentibus vel fulvis, infra luridis vel olivo-brunneis; maculis solitariis 1–5 mm., maculis confluis multo maioribus, areae folii partem dimidia maiorem obtendentibus; mycelio interiori atque exteriori, hyalino ad normam exacto vel abnormi, 2.0–4.40 μ; hyphis exterioribus aliquanto amioribus quam interioribus; conidiophoris hypophyllis, e hyphis mycelii interioris hypostomaticis plus minusve laxis exsistentibus et in fasciculos per stomata emergentibus, vel in mycelio exteriori dissipatis, pronis, septis, hyalinis vel suffuscis (subnigris numquam), 22–65 × 3.9–5.8 μ, ramis alternis, quorum quidam se producunt ut mycelia exteriora fiant; conidiis cylindratis vel subtiliter obclavatis, erectis vel curvatis, ad basim coartatis, coniunctione orbiculata vel truncata, hyalinis, 34–143 × 3.0–4.4 μ, 3–13 septatis, ad septa interdum contractis.

On living leaves of *Pittosporum tobira,* Ait.

TYPE LOCALITY: Baton Rouge, Louisiana.

Type specimens deposited in the herbarium of the Department of Botany, Louisiana State University, and also in the Mycological Collections, Bureau of Plant Industry, Washington, D. C.

DEPARTMENT OF BOTANY,
LOUISIANA AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY, LOUISIANA

[2] The author is indebted to Dr. P G. Moorhead, Professor of Latin, Louisiana State University, for assistance in writing the Latin diagnosis.

MOLLISIA TETRICA, PEZIZA SEJOURNEI, AND THE GENERA PHAEOCIBORIA AND PYCNOPEZIZA

H. H. Whetzel and W. Lawrence White

Von Höhnel (**1918a**), placing the customary emphasis upon spore color as a generic character, erected the genus *Phaeociboria* to provide for an inoperculate Discomycete having brown one-celled ascospores and a prosenchymatous medullary excipulum, yet not referable to *Lambertella* v. Höhn. nor to *Velutaria* Fuckel, the only other genera of the inoperculates known to him to have this combination of characters. His concept of the new genus was formed from a consideration of two species which he took to be identical. These two species had been described originally by Quélet (**1885**) and by Boudier (**1881**) as *Mollisia tetrica* and *Peziza Sejournei* respectively. Von Höhnel's only first hand knowledge of them appears to have been obtained from an examination of two specimens of *M. tetrica,* one distributed by Jaap in his Fungi Selecti Exsiccati No. *501* under the name *Aleurina tetrica* (Quél.) Rehm in litt., and the other by Rehm in his Ascomycetes Exsiccati No. *2153* as *Humaria tetrica* Quél. There is no evidence that von Höhnel had ever examined specimens of *P. Sejournei* Boud. Pending our suggestion of a generic resting place for each of these species later in this paper, we shall use their original designations in the following discussion of the problem.

Through his primary interest in *M. tetrica* von Höhnel was lead to a consideration of *P. Sejournei* largely because both species occur on *Hedera Helix.* After a comparison of the descriptions and the specimens of *M. tetrica* which he had before him, with the descriptions and illustrations of *P. Sejournei,* he concluded that the two are specifically identical notwithstanding his recognition of the following facts, viz.:

(1) that the apothecia of *M. tetrica* are minute, about 1 mm. in diameter, and very dark in color; that they arise from areas in the

leaf parenchyma bordered by a black line, while the apothecia of *P. Sejournei* were described as 2–5 mm. in diameter, light in color, arising from petioles and midribs;

(2) that the ascospores of *M. tetrica* are fusiform to boat-shaped, brown, 20–22 × 3.5–4.5 μ, while those of *P. Sejournei* were described as ovoid oblong, sometimes a little curved, hyaline, 11–13 × 4–5 μ.

His argument in support of his conclusion that the two are specifically identical is based upon his assumption that the size and shape of the ascospores vary greatly in both forms and that the ascospores of the specimens on which Boudier based his description of *P. Sejournei* were immature, hence small and uncolored. As a character indicating close relationship or identity of these two species, von Höhnel emphasizes the significance of peculiar colored structures which he observed in the hymenium of *M. tetrica* and similar structures reported by Boudier in *P. Sejournei.* His interpretation of these structures is without foundation in fact. A careful examination of these colored structures in *M. tetrica* shows them to be merely asci from which the more or less shrivelled ascospores have not been discharged. Boudier (Ic. Myc. *pl. 484*) pictures an ascus with undischarged spores and colored protoplasmic residue. The writers have been unable to find any appreciable number of such asci in the specimens of *P. Sejournei* which they have examined.

In the first of two notes on the matter (**1918a**) von Höhnel discusses a specimen from Rehm's Ascomycetes, No. *2153,* distributed under the name *Humaria tetrica* Quél. [= *Aleurina tetrica* (Quél.) Rehm]. This collection on dead leaves of *Hedera Helix,* had been made by Jaap near Triglitz in Brandenburg in April, 1915. He first questions whether this material really represents a specimen of Quélet's *Mollisia* (*Humaria*) *tetrica,* and then assuming that it does, he further suggests that it may be identical with *P. Sejournei* Boud., citing Boudier's illustration of the latter species in Icones Mycologici, plate 484. Rehm's material appeared to him to be similar to Boudier's fungus in external characters, and like it occurring also on decaying leaves of the ivy. He notes that *P. Sejournei* "kürzere hyaline Sporen, während der ausgegebene Pilz längere, schliesslich rauchgraubraune Sporen hat."

He further points out that the Rehm specimen is inoperculate, having, like *Lambertella* v. Höhn. and *Velutaria* Fuckel, colored spores; that it is in structure a *Ciboria;* and that it, therefore, represents a new genus which he names *Phaeociboria.* He concludes his brief note with the remark, " Er hat *Phaeociboria tetrica* (Qu.) v. H. oder *Phaeociboria Sejournei* (Boud.) v. H. zu heissen, was noch zu entscheiden ist."

In his second more extensive note (**1918b**) entitled " Über *Mollisia tetrica* Quél.," evidently written but a short time after the first, von Höhnel repeats the information given in his earlier note, adds some new observations following his examination of an additional specimen of the fungus taken from the same locality five years later by Jaap (Fung. Sel. Exs., *501*), and commits himself more completely to a belief in the specific identity of *M. tetrica* Quél. and *P. Sejournei* Boud. Since the specific name *Sejournei* (**1881**) has priority over *tetrica* (**1885**) he proposes in his concluding statement the combination *Phaeociboria Sejournei* (Boud.) v. Höhn. for the type species, saying, " Wenn beide Formen zusammengehören, muss der Pilz *Phaeociboria Sejournei* (Boud.) v. H. gennant werden, da Boudier den Pilz zuerst beschrieben hat, im anderen Falle tritt Quélet's Artname in Kraft." Thus it is evident that in his final analysis von Höhnel was still uncertain as to the identity and affinities of *Peziza Sejournei* Boud.

The writers were recently led to a consideration of this case by the thought that *P. Sejournei* Boud. might be a species of the genus *Pycnopeziza* which they had erected in 1938. Discussing this possibility shortly after the publication of the paper on *Pycnopeziza,* and with Boudier's colored illustration of *P. Sejournei* before him, the senior author recalled that he had collected a discomycete of similar aspect in May, 1930 in company with Dr. Eugene Mayor in the Perreux Forest near Neuchatel, Switzerland. Correspondence with Dr. Roger Heim of the Museum National d'Histoire Naturelle, Paris, disclosed the fact that while most of Boudier's herbarium is preserved in good condition at that institution, no specimen of *P. Sejournei* is to be found there. However, examination of the Neuchatel specimen and of the notes taken on the fresh material left no doubt in the writers' minds of its identity with *P. Sejournei* Boud. Although an ascospore cul-

ture from the specimen had been obtained at the time of its collection, this had died out, but a dried specimen of a petri dish culture on potato dextrose agar showing small flat black stromata had been preserved.

On January 11, 1939, a letter was addressed to Dr. Mayor asking him to make if possible another collection of *P. Sejournei* and to send it in fresh condition so that a pure culture of the fungus might again be obtained. Early in May, 1939 a fine collection of this fungus which he had taken on May 7 was received from Dr. Mayor. It was in good condition, but efforts to get cultures failed. However, the specimens provided excellent material for critical study. Failure to find in this material any traces of a conidial stage, which might confirm the "hunch" that *P. Sejournei* may be referable to *Pycnopeziza*, lead to the abandonment of that idea for the time being.

Meantime, the junior writer, scouting the literature and various herbaria for species which might be referable to the genus *Rutstroemia* Karst. emend. Rehm (**1893**) discovered that *Ciboria pachyderma* Rehm is identical with *Pycnopeziza quisquiliaris* (Ellis & Ev.) White & Whetzel. It is interesting in this connection that Rehm (**1893**) in his original description of *Ciboria pachyderma* says in a footnote: "Gehört zu Ciboria trotz kurz und dick gestielter Apothecien. Die auffällig kleinen Sporen trennen die Art von den bekannten; äusserlich nähert sie sich der *Phialea Sejournei* Boud. . . . deren Sporen 8–10 μ lang sind und zwei Oeltropfen enthalten." Later when Rehm (**1915**) transferred *P. Sejournei* Boud. to *Ombrophila* he listed *Ciboria Sejournei* Rehm as a synonym and cited his above quoted observation on the similarity of *Ciboria pachyderma* to that fungus. This seems to be the first and only place where the combination *Ciboria Sejournei* has been made and strongly suggests that Rehm thought his *Ciboria pachyderma* might be identical or at least congeneric with Boudier's species.

With this background of history, observation, and fact before the reader, it is now possible to give consideration to the question of the proper disposition of the species involved in the above discussion.

More fortunate than von Höhnel, the writers have had before

them for comparative study fairly satisfactory material of both *Mollisia tetrica* Quél. and *Peziza Sejournei* Boud. The junior author has examined critically specimens of the former from Jaap's Fungi Selecti Exsiccati No. *501* and from Rehm's Ascomycetes No. *2153,* which are the collections seen by von Höhnel, and he has compared them very carefully with the excellent materials available of *Peziza Sejournei* Boud. This study confirms the accuracy of the original descriptions of the two species and fully establishes that they are specifically distinct. Not only are they distinct specifically, but they would seem best treated at the present time as different generically. It is therefore proposed that the genus *Phaeociboria* v. Höhn. be retained, with *Mollisia tetrica* Quél. as the type species, and that *Peziza Sejournei* Boud. be referred to the genus *Pycnopeziza.*

The validity of colored spores as a generic character in the Helotiaceae may be questioned. Several dark-spored species are at hand which do not appear to be closely related phylogenetically. The closest kin of each will probably be found among hyaline-spored forms. It seems probable in the light of present knowledge of these discomycetes that the brown-spored species may eventually be interpolated among, and considered congeneric with, certain hyaline-spored species to which on the basis of structural characters or on other grounds they would appear to be related.

There are certain structural features of *Mollisia tetrica* Quél. which are common to the species of the genus *Pycnopeziza* White & Whetzel. The genera *Phaeociboria* von Höhn. and *Pycnopeziza* White & Whetzel would therefore seem to be on the best evidence available at present, closely related and in turn allied to *Rutstroemia* Karst. emend. Rehm (type = *Peziza firma* Pers.). A monograph of the latter genus containing some forty species is being prepared by the junior author, and will be ready for publication in the near future; the question of phylogeny and relationships will then be treated at greater length.

The following resumé of the genera *Phaeociboria* and *Pycnopeziza* is presented.

PHAEOCIBORIA von Höhnel, Ann. Myc. **16**: 220. 1918.

Since von Höhnel did not give a formal generic diagnosis the writers present the following:

Apothecia small, stipitate or substipitate, brown or brownish; disc expanded, finally convex, of medium thickness, waxy-cartilaginous when dry; margin circular, obtuse, smooth; receptacle smooth; context light ochraceous brown; in section, differentiated into a thin faintly yellowish hypothecium, a broad colorless medullary layer of loosely interwoven, hyaline, thin-walled hyphae, and a nearly colorless ectal layer of rather compact, parallel, thin-walled hyphae radiating from stipe to margin; paraphyses simple, filiform, colorless; asci clavate-cylindric, opening by a pore; spores narrow-fusoid, brown, one-celled. Type species: *Mollisia tetrica* Quél.

It has not been possible to determine the exact dates of publication of von Höhnel's two notes on *Mollisia tetrica,* in each of which with an equal show of validity he proposes the generic name *Phaeociboria.* Contrary to previous practice[1] by which Fragmente zur Mykologie No. *1123* (**1918b**) is cited as the place of first publication of the genus *Phaeociboria,* the writers have designated the briefer note in Annales Mycologici (**1918a**) because it very evidently was the first article written. In neither article was a formal generic diagnosis presented, and in each of them *Peziza Sejournei* and *Mollisia tetrica* were assumed to be identical. Yet it is clear from the context of either note that the genus was founded primarily on the characters of *M. tetrica;* that mention of *P. Sejournei* in this connection was merely incidental; and that the latter species, in so far as von Höhnel was concerned, is to be disregarded should future investigation prove it a distinct species. Hence, recognition of the genus *Phaeociboria* is not open to criticism on the ground that it was founded on error.

PHAEOCIBORIA TETRICA (Quél.) von Höhnel, Ann. Myc. **16**: 220. 1918.

Mollisia tetrica Quél., Assoc. Fr. Av. Sci. Compte Rendu **14**[2]: 452. 1886.

Humaria tetrica Quél. Ench. Fung. 291. 1886.

Velutaria tetrica Rehm, Rab. Krypt.-Fl. **1**[3]: 647. 1893.

[1] Clements & Shear, **1931**, p. 327; Kanouse, **1935**, p. 75; Nannfeldt, **1932**, p. 298.

Aleurina tetrica Rehm, Verh. Bot. Ver. Prov. Brand. **56**: 77. 1914.

Apothecia solitary, small, substipitate, appearing sessile, attached by a papilla-like central projection, 1–1.5 mm. in diameter; when dry, medium brown to nearly black, more or less concolorous throughout, waxy-cartilaginous, plane to slightly convex; when moistened becoming strongly convex, medium brown, ceraceous-coriaceous, the margin obtuse; in section the hymenium 95–105 μ thick, conspicuous on account of the numerous brown spores in the asci; hypothecium about 40 μ thick, prosenchymatous, pale brownish to nearly colorless, the hyphae compactly interwoven, 3–4.5 μ in diam.; medullary layer broad, hyaline, composed of branching, rather loosely interwoven, septate, thin-walled hyphae, 6–7 μ in diam.; ectal layer not well differentiated, colorless to very slightly brownish, composed of rather compact longitudinally interwoven, thin-walled, short celled hyphae of about the same diameter as those of the medullary layer, not protruding at margin beyond the hymenium; paraphyses simple, colorless, slightly clavate toward the apex, 3–3.5 μ in diam.; asci clavate cylindric 100–110 $\times$ 10–12 μ; spores one-celled, biseriate, at first hyaline, narrowly fusoid with 4–6 irregular oil globules, soon becoming cinnamon brown, narrow oblong-fusoid, slightly inaequilateral, 18–24 $\times$ 4–5.5 μ, the ends obtuse.

HABITAT: On *Hedera Helix;* reported on dead stems and leaves; noted by the writers only on the leaves, arising directly from the leaf blade, unassociated with the veins.

TYPE LOCALITY: Vosges, France.

DISTRIBUTION: Known only from France and Germany. Apparently rare. "Automne" (Quélet), April.

France: Vosges (Original description of Quélet).

Germany: Triglitz (Prignitz), Prov. Brandenburg, April 15, 1911;[2] a second collection from same locality, col. Jaap, April, 1915, Rehm, Ascom. Exs. *2153.*[3]

ILLUSTRATIONS: Quélet, Assoc. Fr. **1885**, pt. 2, *pl. 12, f. 27.* 1886.

EXSICCATI: Jaap, Fung. Sel. Exs. *501;* Rehm, Ascom. Exs. *2153.*

[2] Specimen examined, from Fairman Herb. in Dept. Plant Pathology, Cornell University.

[3] Specimens examined, from Farlow Herb. (no apothecia found), and from U. S. D. A., Path. & Mycol. Collections (one apothecium present).

PYCNOPEZIZA White & Whetzel, Mycologia **30**: 187. 1938.

This genus was founded to include species having certain distinctive apothecial characters and a highly characteristic conidial stage. An adequate generic diagnosis was presented in the previous paper (White & Whetzel, **1938**) and need not be repeated here. Type species: *P. sympodialis* (Bub. & Vleug.) White & Whetzel.

Pycnopeziza Sejournei (Boud.) comb. nov.

Peziza Sejournei Boud. Bull. Soc. Bot. France **28**: 94. 1881.
Phialea Sejournei Boud. Icon. Myc. **4**: 282. 1905–1910.
Ciboria Sejournei Rehm, Ber. Bayer. Bot. Ges. **15**: 246. 1915.
Ombrophila Sejournei Rehm, Ber. Bayer, Bot. Ges. **15**: 246. 1915.
Phaeociboria Sejournei von Höhnel, Ann. Myc. **16**: 220. 1918.

Apothecia solitary, substipitate, erumpent, at first turbinate, reaching a diameter of 2–6 mm., thick, fleshy; externally pale ochraceous brown, furfuraceous; stipe short, thick, often scarcely distinct, broadened rather gradually above into the receptacle; margin circular, obtuse; hymenium patelliform, finally plane or slightly convex, yellowish- or reddish-brown in color; in drying changing color and shape but little, the hymenium becoming very dark reddish brown and slightly concave, the stipe-like basal portion rather deeply lacunose-wrinkled, when broken showing a thick pale brown or deep cream color and a more or less powdery context. In section showing a narrow, yellowish, poorly defined hypothecium about 30 μ thick, composed of narrow compactly interwoven hyphae; medullary excipulum broad, colorless, homogenous, the hyphae rather compactly interwoven, thin-walled, 4–7 μ in diam.; ectal layer not well defined, faintly yellowish, composed of compactly and longitudinally interwoven hyphae which on the outer surface and especially toward the stipe are broken into thin-walled isodiametric cells, 10–15 μ in diameter. Paraphyses simple, scarcely or not at all enlarged above, colorless, 3.5–4.5 μ in diameter; asci cylindric above, narrowed below, 95–110 $\times$ 7.5–8.5 μ; spores hyaline, 1-celled, sometimes with a small oil globule in each end, narrow ellipsoid, 9–13 $\times$ 4.5–5 μ, obliquely or more or less irregularly uniseriate.

HABITAT: On *Hedera Helix;* dead stems, petioles, and basal portions of leaf veins.

TYPE LOCALITY: Blois Forest, France.

DISTRIBUTION: Known only from central Europe. Evidently not common. April and May.

France: Blois Forest (Original description of Boudier), May; Presle, April 23, 1910 (Specimen examined from Patouillard Herb. in Farlow Herb., Harvard Univ.).

Germany: Aschaffenburg. Report by Rehm (**1915**, p. 246) based on a collection by A. Ade. Von Höhnel suggests that Rehm's specimen may be incorrectly named.

Switzerland: Neuchatel, Perreux Forest, May 11, 1930, H. H. Whetzel, Cornell Univ. Pl. Path. Herb., *27980;* Perreux Forest, May 7, 1939, E. Mayor, Cornell Pl. Path. Herb., *28442.*

ILLUSTRATIONS: Boud., Bull. Soc. Bot. Fr. p. 94, *pl. 2, f. 4,* 1881; Boud., Icon. Myc. **3**: *pl. 484,* 1905–10.

While no conidial stage has been found in the material available for examination, the characteristic apothecial features nevertheless seem to indicate a close relationship with the species previously discussed by the writers (**1938**) as *Pycnopeziza quisquiliaris,* and for which a new combination must now be proposed (see below). From the latter it differs only in having slightly larger spores, a considerably thicker medullary excipulum, thicker stipe, and in its occurrence on *Hedera Helix.* The writers had hoped for the discovery of a conidial stage corresponding to those of the species previously included in *Pycnopeziza* which would of course have fully confirmed the proposed disposition of this very interesting species.

Pycnopeziza pachyderma (Rehm) comb. nov.

Ciboria pachyderma Rehm, Rab. Krypt.-Fl. 1^3: 758. 1893.

Cyathicula quisquiliaris Ellis & Ev. Proc. Acad. Phila. **1893**: 451. 1894.

Pycnopeziza quisquiliaris White & Whetzel, Mycologia **30**: 192. 1938.

The new combination is necessitated by the discovery of the fact that *Ciboria pachyderma* Rehm is synonymous with *Cyathicula quisquiliaris* Ellis & Ev. and has priority over the latter by one year. Under the name *Ciboria pachyderma* the species has been distributed in Sydow's Mycotheca Marchica as Nos. *1269, 3480,*

3777, 3955, and *4157,* and in Rehm's Ascomycetes as No. *758;* all of these were taken in Germany. Examination of these specimens together with reports of the species under the same name by Rehm (**1893**, p. 758), Dodge (**1914**, p. 1033), and Velenovský (**1934**, **1**: 217; **2**: *pl. 22, f. 11*) calls for no modification of the description already presented (White & Whetzel, **1938**). However, certain data concerning distribution, substrata, etc. may be briefly summarized. The species is now known in North America from Iowa, New York, West Virginia, and Wisconsin, and in Europe from Czechoslovakia and Germany. The substrata include decaying leaves of *Alnus rugosa, Prunus serotina, Quercus, Salix,* and *Spiraea,* buds of *Acer rubrum,* and leaves of undetermined species (numerous collections). All of the North American and most of the European collections were made during the spring months of April, May, and June; exceptions are Sydow, Mycotheca Marchica Nos. *3480, 3955,* and *3777* taken in Sept., Oct., and Nov. respectively. Specimens of these were examined at the New York Botanical Garden more than a year ago and are not at hand now for reexamination. It is possible that they represent minor variations of the species, for in the experience of the writers most species of the Ciborioideae have a restricted seasonal period of apothecial production.

PYCNOPEZIZA PACHYDERMA var. DEPRESSA Vel. Monogr. Discom. Boh. **1**: 217. 1934.

"Apoth. sublobata, ochracea, centro infundibuliformi-impressa, stipite concolor. Ad folia quercina in valle prope Zahorany maio 1927 leg. Cejp."

The above description by Velenovský, though entirely inadequate for certain recognition of the form, is strongly suggestive of *Pycnopeziza sympodialis.* However, until Velenovský's specimen can be critically examined it seems best to leave it where the author originally placed it.

PYCNOPEZIZA SYMPODIALIS (Bub. & Vleug.) White & Whetzel, Mycologia **30**: 190. 1938.

Acarosporium sympodiale Bub. & Vleug., Ber. Deutsch. Bot. Ges. **29**: 385. 1911.

In the previous publication by the writers, three types of fruiting

structures were described as occurring in the life cycle of *P. sympodialis*. Only North American material was then at hand. The conidial fungus *Acarosporium sympodiale*, known only from the type collection taken in Sweden, was assumed to represent the conidial stage of this pleomorphic species, and this with little misgiving since the characterization by Bubák (**1911**) of this very distinctive form was wholly adequate. When it was learned recently that the Bubák Herbarium is now at the Brooklyn Botanic Garden, and not in Europe as the writers had formerly assumed, the type specimen was borrowed for study. Only the conidial stage is present in the small amount of leaf debris in the type packet. The pycnidia are fairly numerous and are in excellent condition. Agreement of the pycnidial stage from the North American material with this European type is so wholly complete as to remove all doubt of their specific identity. A second collection in the Bubák Herbarium which bears the same data as the type, except that it was taken a year later, is rather unsatisfactory for study, but enough of the conidial stage was found to make identification with the type positive.

The writers wish to express their thanks to Professor H. M. Fitzpatrick for critical reading of the manuscript; to Dr. D. H. Linder, Farlow Herbarium, Harvard University, Dr. G. M. Reed, Brooklyn Botanic Garden, Dr. F. J. Seaver, New York Botanical Garden, and Mr. John A. Stevenson, U. S. Dept. of Agriculture, for making available for examination specimens from their respective herbaria; to Dr. Roger Heim of the Paris Museum for information concerning Boudier collections of *Pycnopeziza Sejournei;* and to Dr. E. Mayor of Neuchatel, Switzerland, to whom they are especially indebted for furnishing fresh material of *P. Sejournei.*

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, NEW YORK

LITERATURE CITED

Bukák, Fr. Ein neuer Pilz mit sympodialer Konidienbildung. Ber. Deutsch. Bot. Ges. **29**: 381–385. 1911.

Clements, F. E. & Shear, C. L. The genera of fungi (New York). 1931.

Dodge, B. O. Wisconsin Discomycetes. Trans. Wis. Acad. **17**[2]: 1027–1056. 1914.

Höhnel, Franz von. Rehm: Ascomycetes Exs. Fasc. 56 & 57. Ann. Myc. **16**: 209–224. 1918a.

——. Über *Mollisia tetrica* Quélet. Fragmente zur Mykologie, Mitt. XXII. Sitz-ber. Akad. Wien **127**: 43–46. 1918b.

Kanouse, Bessie B. Notes on new or unusual Michigan Discomycetes, II. Papers Michigan Acad. Sci. **20**: 65–78. 1935.

Nannfeldt, J. A. Studien über die Morphologie und Systematik der nichtlichenisierten inoperculaten Discomycetes. Nova Acta Regiae Soc. Sci. Upsal. 4: **8**[2]: 1–368. (Upsala) 1932.

Quélet, L. Quelques espèces critiques ou nouvelles de la flore mycologique de France. Assoc. Fr. Av. Sci. Compte Rendu **14**[2]: 444–453. 1886.

Rehm, H. Ascomyceten: Hysteriaceen und Discomyceten. Rab. Krypt.-Fl. **1**[3]: 705–768. 1893.

——. Zur Kenntnis der Discomyceten Deutschlands, Deutsch-Oesterreichs und der Schweiz. Ber. Bayer. Bot. Ges. **15**: 234–254. 1915.

Velenovský, Jos. Monographia Discomycetum Bohemiae, 2 vol. (Prague)). 1934.

White, W. Lawrence & Whetzel, H. H. Pleomorphic life cycles in a new genus of the Helotiaceae. Mycologia **30**: 187–203. 1938.

A FURTHER REPORT ON THE UREDINALES OF COLOMBIA[1]

FRANK D. KERN AND H. W. THURSTON, JR.

Several comprehensive accounts of the rust-flora of Colombia have been published. The first was a most excellent paper by Dr. Eug. Mayor in 1913 entitled "Contribution à l'étude des Uredinées de Colombie" (Mem. Soc. Neuch. Sci. Nat. **5**: 442–599). This paper was well illustrated and in it the author set forth the character and standing of the rust species with clarity and exactness. It has been the foundation for all subsequent studies of Colombian rusts. Not only that, it has been invaluable in all investigations of West Indian and South American Uredinales.

During the years 1926–29 Messrs. C. E. Chardon, J. A. B. Nolla, and R. A. Toro made extensive collections of fungi in Colombia. The rust specimens were placed in the hands of F. D. Kern and H. H. Whetzel. As a result of their studies a section on Uredinales was contributed by them in 1930 to "Mycological Explorations of Colombia" (Jour. Dept. Agric. Puerto Rico **14**: 301–348).

Three years later Kern, Thurston, and Whetzel published an "Annotated Index of the Rusts of Colombia" (Mycologia **25**: 448–503). In this paper an attempt was made to include all of the species of rusts known to occur in the country. This 1933 list presented many changes and additions when compared with the 1930 list. In explanation the following statement was made: "Changes may be due to the discovery of additional spore-forms, or they may be due to additional characters discoverable from the specimens but not from the descriptions, or to mistaken identity of hosts, or to some combination of these or other new data."

[1] Contribution from the Department of Botany, The Pennsylvania State College, No. 131. Publication authorized on February 24, 1940 as paper No. 958 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

Again we have changes and additions. There are several changes in names which seem necessary. We are abandoning the name *Puccinia epiphylla* and taking up *Puccinia Poae-sudeticae* as explained in the note. The *Geranium* rust has become *Puccinia unilateralis*. As a result of the discovery of teliospores it has been taken from the form-genus *Uredo*. In the 1930 paper an aecium on *Capsicum* was described as *Aecidium Capsici*. Teliospores have been found which complete the life cycle. Since there is a microcyclic *Puccinia* under the specific name *Capsici* a new name must be provided. We are proposing *Puccinia capsicicola*. Numerous collections of rust on species of the genus *Inga* have been made in Colombia. The 1930 and 1933 papers referred all collections to *Ravenelia Ingae*. The most recent opinion is that two species are present—one to be called *Bitzea Ingae* and the other *Uredo Ingae*. *Uredo jatrophicola* now becomes *Phakopsora jatrophicola* through the discovery of teliospores.

Although not much mycological exploration has been carried out in Colombia since 1933 we have had for study approximately fifty specimens. These have been collected chiefly by Dr. Carlos E. Chardon. We are now reporting that eleven species are added to the number reported in 1933, bringing the total up to 226. Two of these are described as new. One species was added in 1939 by the description of the new genus *Chardoniella* (Kern, Mycologia **31**: 375). Another species appears because of the separation of *Ravenelia Ingae* into two species. This means that seven species are now recognized for the first time from Colombia owing to the collections of Chardon in 1936 and in 1937. Two of these have not been reported previously from South America.

AECIDIUM CAPSICI Kern & Whetzel.
See *Puccinia capsicicola*.

BITZEA INGAE (Sydow) Mains, Mycologia **31**: 38. 1939.
Maravalia Ingae Sydow, Mycologia **17**: 257. 1925.

In the 1933 paper nine collections were referred to *Ravenelia Ingae*. Of these the following are now regarded as belonging to the species *Bitzea Ingae:*

On *Inga adenophylla* Pittier, *Toro 190*.

Inga edulis Mart., *Chardon 110.*
Inga cf. *ingoides* (Rich.) Willd., *Mayor 311.*
Inga spuria H. & B., *Archer H-34.*
Inga sp., *Chardon & Nolla 448; Chardon 90.*

These specimens all have present one-celled spores with markings consisting of prominent longitudinal ridges and less pronounced cross ridges. This spore-form has been described twice in the genus *Uromyces* as telia (*U. ingicola* P. Henn. and *U. porcencis* Mayor) and once in the genus *Ravenelia* as uredinia (*Ravenelia Whetzelii* Arth.). Without doubt these striately marked spores are urediniospores and according to Mains they are a part of a life cycle of his species *Bitzea Ingae.*

There is present in Colombia a spore-form on *Inga* with echinulate markings. According to Mains this form "may possibly be the secondary uredinia of *Bitzea Ingae* but the evidence appears to be against it." We are following Mains in listing collections of this sort under *Uredo Ingae.*

CHARDONIELLA GYNOXIDIS Kern, Mycologia **31**: 375. 1939.

On *Gynoxis* sp., Cerro Monserrate pr. Bogotá, alt. 2750 m., March 12, 1937, *C. E. Chardon 829.*

Known only from the type specimen.

MAINSIA CUNDINAMARCENSIS (Mayor) Jackson, Mycologia **23**: 114. 1931.

On *Rubus* sp., Peña negra, above Facatativa, March 28, 1937, *C. E. Chardon 856.*

Our specimen agrees so well with Mayor's original collection that we have no hesitation in referring it to this species. Jackson has called attention to the fact that the urediniospore wall is distinctly thickened at the apex although this was not mentioned by Mayor. Jackson said the markings are much more prominent at the apex than at the base. We think he should have added that the apical markings are of a ridge-like nature giving the effect of striations. The species is known also from Venezuela and Ecuador.

PHAKOPSORA COLUMBIANA Kern & Whetzel. Jour. Dept. Agric. Puerto Rico **14**: 304. 1930.

On *Croton gossypiifolius* Vahl, Girmania, Viteletes, alt. 1025 m., April 11, 1937, *C. E. Chardon 861.*

This species was described from Ibagué, Colombia, where it was collected by C. E. Chardon, June 20, 1929, no. *555.* The original description states that the urediniospores are *23–27* × *26–31* μ, the walls cinnamon brown, echinulate, *2* μ or more thick. That description does not cover the range of these spores. A restudy reveals many spores of large size, *24–32* × *29–42* μ, with walls from 3–6 μ thick, yellowish, and strongly aculeate. It is possible that these lighter thicker walled spores are immature. Dr. G. B. Cummins has recently sent us a specimen from Trinidad, said to be on the type-host, *Croton gossypiifolius* Vahl, which agrees perfectly with the type as regards the urediniospores.

PHAKOPSORA JATROPHICOLA (Arth.) Cummins, Bull. Torrey Club **64**: 43. 1937.

Specimens previously cited under *Uredo jatrophicola* are now referred to the genus *Phakopsora.* Dr. Cummins found telia on a specimen from Lower California. He says the rust is common in tropical North America. It is known also from Colombia, Brazil, and Venezuela in South America.

PUCCINIA ANTHEPHORAE (Sydow) Arth. & Johnston, Mem. Torrey Club **17**: 137. 1918.

On *Anthephora hermaphrodita* (L.) Kuntze, Finca Santander, Cucuta, December 7, 1936, *C. E. Chardon 815.*

This species has heretofore been known only from the West Indies. Arthur and Johnston commented that it "is doubtless widespread, although it may not be abundant, throughout the West Indies, as the host is a wayside weed." It is interesting that no other collections have turned up from the West Indies and this is, so far as we know, the only additional report of the species.

Puccinia capsicicola nom. nov.

Aecidium Capsici Kern & Whetzel, Jour. Dept. Agric. Puerto Rico **14**: 341. 1930.

Not *Puccinia Capsici* Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: 501. 1913.

A reexamination of the type specimen has revealed the presence of a few telia. They arise in the old aecial cups in such a way that there can be no question of their relationship. The species is therefore a *Puccinia* of the opsis-type. We would describe the telial stage as follows:

Telia in the old aecia, blackish; teliospores broadly ellipsoid, 18–23 × 37–55 μ, rounded above and below, slightly constricted at the septum, the wall chestnut-brown, smooth, 3–4 μ thick, slightly thicker at apex, 5–6 μ; pedicel short.

This species is similar to *Puccinia paulensis* Rangel (Archiv. Jard. Bot. Rio de Janeiro **2**: 70. 1918) but differs in having teliospores that are narrower and longer and aeciospores that are smaller and thicker walled.

PUCCINIA EPIPHYLLA (L.) Wettst.

See *Puccinia Poae-sudeticae* (Westend.) Jørstad.

PUCCINIA GNAPHALII (Speg.) P. Henn. Hedwigia Beibl. **41**: 66. 1902.

On *Gnaphalium spicatum* Lam., LaVirginia, camino del Ruiz, alt. 2700 m., December 25, 1936, *C. E. Chardon 839*.

This species is known from the Southern United States, Central America, and several South American countries but we have found no previous report from Colombia.

PUCCINIA HELIOTROPII Kern & Kellerm. Jour. Myc. **13**: 23. 1907.

On *Heliotropium indicus* L., Finca Santander, Cucuta, December 7, 1936, *C. E. Chardon 814*.

The first report of this species from Colombia. It is otherwise known from Guatemala and Venezuela. We know of a total of only five collections from all localities.

Puccinia immensispora sp. nov.

Pycnidiis epiphyllis, paucis, in maculis decoloratis teliis objectis, profunde insidentibus, conspicuis, punctiformibus, magnis, usque 300 μ diam., periphysibus brevibus.

Teleutosoris hypophyllis, gregariis vel confluentibus, in greges circinatim dispositis 2–3 mm. diam., nudis, ceraceis, dein ob germinationem pulverulentibus, flavis; teleutosporis oblongis vel anguste oblongo-ellipsoideis, 31–40 × 125–170 μ, supra rotundatis vel angustatis, infra angustatis, ad septum leniter constrictis; tunica hyalina vel tincta, 3–5 μ cr., leve; pedicello lato, sporam aequante vel breviore.

On *Diplostephium* sp. (?), paramo de Chipaque, alt. 1000 m., April 1, 1937, *C. E. Chardon, E. Perez Arbelarz, H. Garcia Barrios 840.*

The outstanding characteristic of this species is the large size of the teliospores. Sydow in his Monographia Uredinearum, vol. 1, p. 190, said that *Puccinia nervincola* Lagerh. from Ecuador had the largest teliospores of all the species of *Puccinia.* The size given for that species is 14–22 × 135–162 μ. Our species has spores that are much broader and there are other differences such as the apical thickness of the walls. Sydow has recently described two other species of *Puccinia* with large spores, both from Ecuador. Neither of them equal our species in size of the spores and there are other differences as well.

All of Sydow's species are on undetermined hosts. Our specimen is not without some doubt. Dr. S. F. Blake has said that it is probably *Diplostephium.*

Puccinia liabicola sp. nov.

Pycnidiis epiphyllis, paucis, gregariis in maculis decoloratis, 2–3 mm. diam., profunde insidentibus, obscure bruneis, conspicuis, subepidermalibus, globoidiis 175–190 μ diam., periphysibus brevibus.

Teleutosoris hypophyllis, in maculis dense aggregatis et pycnidiis objectis, mox nudis, rotundatis, cinnamomeo-brunneis ob germinationem cinerascentibus, 0.3–0.4 mm. diam.; epidermide rupta inconspicua; teleutosporis oblongis vel oblongo-clavatis, infra angustatis, supra rotundatis, leniter constrictis, 23–27 × 60–85 μ; tunica tenui ca. 1 μ, hyalina vel tincta, leve; pedicello hyalino, lato, sporam dimidiam aequante.

On *Liabum* sp., Tequendama, December 13, 1936, *C. E. Chardon 818.*

A microcyclic species, resembling *Puccinia Liabi* in some ways,

but differing in the presence of pycnia and in certain well defined spore characters. In *Puccinia Liabi* the spores are brownish and 14–21 × 40–59 μ. Here they are colorless, or slightly tinted, and 23–27 × 60–85 μ. In the former the apical wall is thickened up to 4 μ, here the wall is uniformly thin.

PUCCINIA OBLONGULA Jackson & Holw. Mycologia **18**: 145. 1926.

On *Rynchospora* sp., Tequendama, December 13, 1936, *C. E. Chardon 824.*

There are present only urediniospores. They agree so well with the type that we have no hesitation in making this determination. This appears to be the first report outside the type locality which is in Ecuador.

PUCCINIA POAE-SUDETICAE (Westend.) Jørstad, Mag. Naturv. **70**: 325. 1932.

On *Poa annua* L., gardens at El Colegio, near Madrid, March 29, 1937, *C. E. Chardon 850.*

Alopecurus aequalis Sobol., gardens at El Colegio, near Madrid, March 28, 1937, *C. E. Chardon 858.*

In our previous report (Mycologia **25**: 469, 1933) we pointed out that the name *Puccinia Poae-sudeticae* seemed to be the proper name for this species but actually we used instead the name *Puccina epiphylla.* We are now convinced that the latter name refers to a northern species which does not occur in this region at all. It has no or very inconspicuous paraphyses in the uredosori. All of our specimens from Colombia have paraphyses and belong to *Puccinia Poae-sudeticae.* Besides the two specimens here referred to, there are also Chardon's numbers *616, 628,* and *631,* and Toro's number *410.* We should add that many others have used the name *Puccinia Poarum* for this species but that is to be regarded as an erroneous use of the name. *Puccinia Poarum* Nielson is synonymous with *Puccinia epiphylla* (L.) Wettst.

PUCCINIA POLYMNIAE Jackson & Holw. Mycologia **24**: 167. 1932.

On *Polymnia pyramidalis* Triana, ravine between Monserrate and Guadalupe, above Bogotá, March 12, 1937, *C. E. Chardon 825.*

This is a species with an unusual form of urediniospores. They are vertically flattened with four subequatorial pores. It has been previously reported from Ecuador and Bolivia.

PUCCINIA UNILATERALIS (Arth.) Cummins, Bull. Torrey Club. **67**: 67. 1940.

On *Geranium caucense* R. Knuth, La Virginia, camino del Ruiz, alt. 2700 m., December 26, 1936, *C. E. Chardon 811.*

In a recent paper Cummins has transferred *Uredo unilateralis* to *Puccinia.* He found 2-celled teliospores on a specimen from Mexico. The teliospores are peculiar in that they are sessile on a cellular hymenium. In an earlier paper (Kern & Whetzel, Jour. Dept. Agric. Puerto Rico **14**: 347, 1930) we reported *Uredo unilateralis* on *Geranium hirtum* from Colombia (*Chardon 596*). Teliospores have been found on this specimen and although they differ from the Mexican specimen in being narrower and in having a thinner apical wall Cummins expresses the opinion that this specimen probably belongs to the same species. The pycnia, aecia, and uredinia agree.

RAVENELIA INGAE (P. Henn.) Arth.
See *Bitzea Ingae* (Sydow) Mains.
Uredo Ingae P. Henn.

UREDO INGAE P. Henn., Hedwigia Beibl. **38**: 69. 1899.
On *Inga* sp., *Chardon & Nolla 437, 490; Archer H-155.*
See note under *Bitzea Ingae.*

UREDO JATROPHICOLA Arth.
See *Phakopsora jatrophicola* (Arth.) Cummins.

UREDO NIDULARII P. Henn. Hedwigia Beibl. **37**: 206. 1898.

On *Guzmania* sp., ravine between Monserrate and Bogotá, March 12, 1937, *C. E. Chardon 832.*

Hennings described this species on *Nidularium* from Brazil. A specimen was issued in Rab. Fungi Eur. *4340.* Through the kindness of Dr. D. H. Linder, of the Farlow Herbarium, we have had

the Rabenhorst specimen for comparison. Our specimen agrees well.

UREDO TRINIOCHLOAE Arth. & Holw. Am. Jour. Bot. **5**: 538, 1918.

On *Triniochloa stipoides* (H. B. K.) Hitchc., hills about Facatativa, March 28, 1937, *C. E. Chardon 854*.

This species was described from Guatemala where it was collected in 1915. This is, so far as we know, the second collection.

THE PENNSYLVANIA STATE COLLEGE,
STATE COLLEGE, PA.

"MUTATIONS" IN ASPERGILLUS NIGER BOMBARDED BY LOW VOLTAGE CATHODE RAYS[1]

ROY M. WHELDEN

(WITH 4 FIGURES)

The study of variations or of true mutations in organisms exposed to various forms of ionizing radiation has been carried forward actively ever since suitable sources of these various radiations were developed. Ultraviolet light, X-rays, high velocity cathode rays, radium, and neutrons have all been used more or less extensively. The greater number of variations that has been recorded, however, have occurred in higher animals and plants. Fungi have received relatively little attention. Nadson and Phillipov (**7**) have reported mutations in *Mucor genevensis* under X-ray exposure. The same authors (**8**, **9**) later recorded the occurrence of permanent modifications in X-rayed yeasts. Other workers, notably Holweck and Lacassagne (**6**) and Nadson and Rochlin (**6**) abroad and Wyckoff and Rivers (**12**), in this country, have investigated modifications in yeasts exposed to ionizing radiations. Work on modifications in *Aspergillus niger* following the exposure of spores to high voltage cathode rays has been done by Haskins and Moore (**5**). Dodge (**3**) noted that X-raying ascospores in *Neurospora tetrasperma* has lethal effects. Dickson (**2**) showed that ultraviolet light, X-rays, and heat could produce saltations in *Chaetomium cochliodes* and other fungi, the saltants being distinguished from the parent strains by color differences, by changes in number and size of perithecia, or complete lack thereof. Quite recently Emmons and Hollaender (**4**) reported that dermatophyte spores exposed to ultraviolet light gave rise to several mutations.

It is the purpose of the present paper to describe a series of

[1] Contribution from the Haskins Laboratories and from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, No. *183*.

variations occurring in plants of *Aspergillus niger* arising from spores subjected to conditions to which fungus spores have not been previously submitted; namely, bombardment in vacuum by low-density beams of low-velocity electrons, comparable in their density and range to the secondary electrons released in spore tissues under X-ray bombardment. The velocity of these electrons was so adjusted that the maximum ionization occurred in the region of the spherical, centrally located nucleus of the spore.

This work grew out of a three year study of the inactivation effects of low velocity cathode rays upon spores of *Aspergillus niger* in vacuum. Dry spores of the organism were spread, in a single uniformly distributed layer, one spore deep, on highly polished chromium-plated brass slides and mounted on a drum within the raying box of a cathode ray tube. The tube was sealed and evacuated, and the spores exposed to the electron beam under carefully controlled conditions, so that both velocity of electrons and total incident dosage to the spore-bearing plate were accurately known. The tube was then returned to atmospheric pressure, and the spores "printed" off into Petri dishes of potato-maltose agar and cultured and studied under standard conditions. This work has been described elsewhere (**1**). Except in those cases in which irradiation resulted in the death of every spore, single spore cultures were made for further study of as many of the irradiated spores as possible after each "run." In the early work very low speed electrons were used, of insufficient velocity to penetrate to the spore nucleus, or, in some cases, of sufficiently great velocity so that the ionization tracks ended well beyond it. The maximum ion concentration therefore lay in the cytoplasm. Among all the cultures studied from irradiations of this type, only one variation appeared, distinguished by its brown spores (**11**). Cultures of many successive generations of this variant have been made, without any apparent alteration.

When examination was begun of plants of *Aspergillus* arising from spores bombarded by electrons of 11.52 electron kilovolts energy, it at once became apparent that this voltage range was a rather critical one in the production of variations. Effects occurred here which were recognizable very early in the development of the spores. At all lower voltages the spores which had been

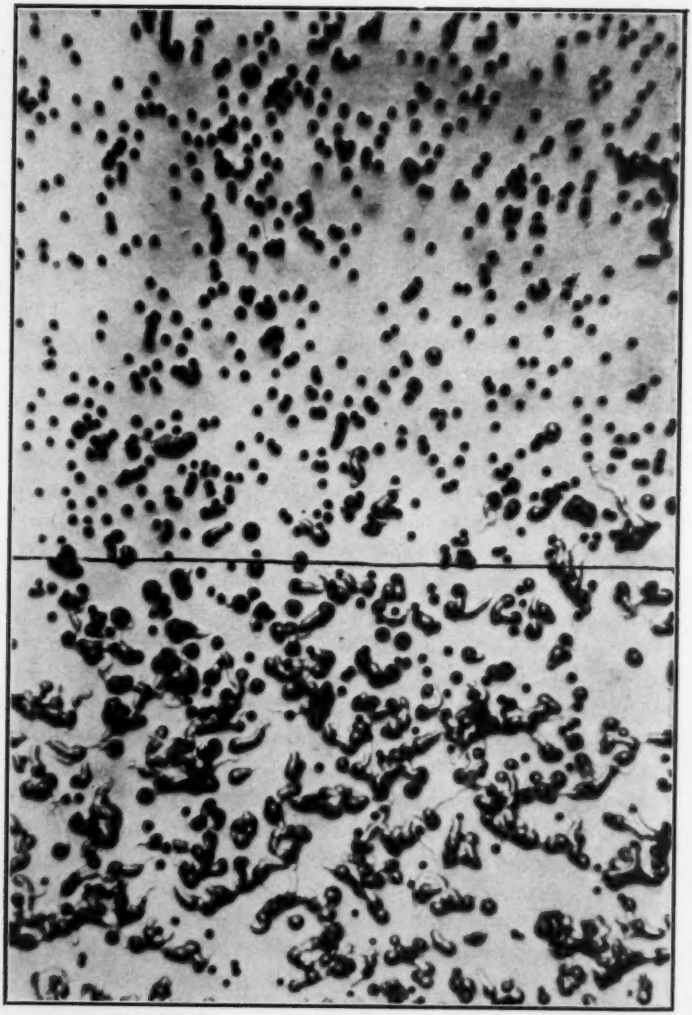


FIG. 1.

irradiated could be positively identified as "living" or "dead" at the end of approximately eight hours. "Living" spores were considered to be those which developed distinct germ tubes, well advanced at the end of the eight hour period. The obvious difference observable between "living" and "dead" spores at certain voltages and dosages is indicated in figure 1, which shows the appearance of spores on each side of the edge of the rayed band under heavy dosage and fairly low voltage.

It was found that when electron beams in the vicinity of 12 kv. were used, the clear-cut distinction between "living" and "dead" spores vanished. Under a calculated dosage of 9.17×10^{-5} ergs per spore at this voltage, there were obtained all degrees of germination, from spores showing germ tubes as far advanced as those of the controls to spores showing only the very slightest swelling at the end of the culture period. The determination of the 50 per cent survival ratio point was exceedingly difficult with these markedly indefinite or transitional spore effects. The wide range of stages of development between the active and the inactive condition is well shown in figure 2, where the controls are at the left half of the photograph, and the spores irradiated under the conditions described are at the right.

Another anomaly in cultures grown from spores irradiated under these conditions became apparent later in their development. As in all previous cases, the irradiated spores were transferred from the original culture to new agar plates for further study. At first single spore transfers were made, but later groups of 15 to 20 spores were transferred to the new agar surface. Similar transfers of control spores were made at the same time. A small transfer chamber was used throughout, sterilized as thoroughly as possible by steaming and washing all surfaces used with a solution of bichloride of mercury. After transfer, the dishes were set aside to permit development of the cultures until sporulation was well advanced. Since the survival ratio of the material was something under 50 per cent, not more than 10 of the 15–20 spores so transferred ordinarily developed.

After the cultures had "matured" so that spore formation was actively in progress, many showed "sector" formation (FIG. 3). Since not one of the control dishes showed such sector formation,

it seemed reasonable to attribute this result to irradiation. It also seemed logical to assume that each of these modified "sectors" arose from a single spore.

As soon as spores appeared in the modified sectors, subcultures were made from spores of each sector, and also from bits of mycelium from the edges of the cultures. These subcultures were grown until spore formation was well advanced when the process was repeated. In all, eight successive transfers were made from each of the original sectors. When the eighth transfer was made, one was also made from a spore of the original sector culture. The two resulting cultures were carefully compared, to check further variations which might have occurred after subculturing was begun. In all cases here reported no evident differences could be observed. It seems permissible to assume, therefore, that variation is fixed.

Specimens of each of the variants obtained in these early radiation runs were preserved in sealed tubes, all the others being destroyed when the eighth transfer had matured. Several weeks later the earlier work was carefully repeated. Four distinct variants had been obtained from the first run. The next run gave ten variant sectors. Certain of these presented features which made special treatment necessary. Two of these will be mentioned later. The third run gave four variants. The fourth run, from which over two hundred single spore transfers were made, gave five variants, two of which failed to survive transfers. One of the permanent variants, appearing in this run for the first time, was very noticeable because of its coarseness. Two later runs each gave about the same numbers of variants. At the end of the fifth run it was apparent that some of the variants of each run were quite like those of previous runs.

Comparative examinations show that most of the variants are referable to five distinct types. One of these types when its spores are fully mature, has a color varying from "avellaneous" to "wood brown" (Ridgway Color Standards and Nomenclature). Another variant at the same stage of growth has a color corresponding to "Saccardo's umber." A third is "mummy brown." In the case of these three variants no attempt will be made to describe in detail the gradual change in color from white through

various shades of pink to the final brown. The differences also become negligible when the culture begins to dry with age, when they all look practically alike. The distinct colors reappear when

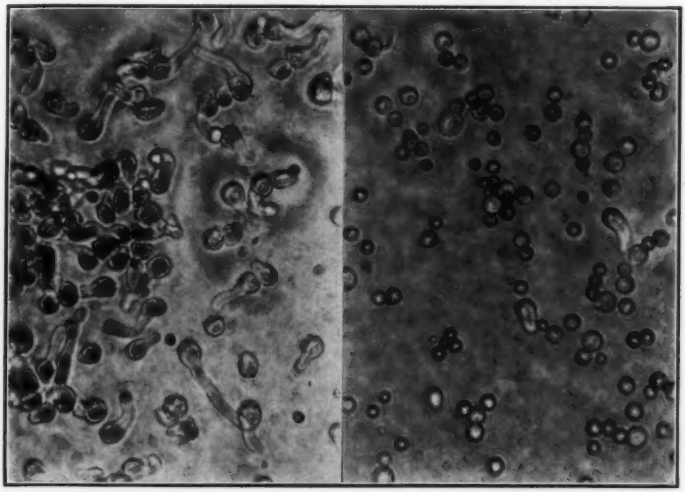


FIG. 2.

a new culture is grown to maturity. The fourth variant, which has occurred only twice, is much more striking than any of the others. Early in its development the mycelium of this form shows a brilliant "citron yellow" color. As the culture matures, its older portions become "pyrite yellow" in color. The mature spores are brownish in color and much darker than the mycelium. The last variant to be mentioned is much larger than any of the others from the earliest stages of its growth. It grows much faster than either the parent *Aspergillus* or any other variant and forms a much more luxuriant mycelium than the others. Spore formation, however, occurs later here and the sporangiophores are not as numerous. The spores of this "giant" variant have the same black color as those of the type of the species and in size are larger, having an average diameter of 4 μ, in contrast to the 3.5 μ of the species and all the other variants. This "giant" form has a much coarser appearance than the others (FIG. 3, central figure of the lower row).

Two other variants, mentioned earlier in this paper, may well be considered at this point. One of these was characterized by the pale grayish appearance it presented when mature. Microscopic examination of the first transfer showed its spores all more or less shriveled and abortive. Furthermore, the number of chains of spores growing on each vesicle was much less than the normal for the species. This variant did not long survive. The second variant was characterized by a complete absence of spores. Subcultures were repeatedly made from the mycelium and were grown under a variety of conditions, but none led to spore formation. Finally this form was discarded.

It will be noticed that, except in the "giant" and the sterile forms, the only character which distinguishes these variants from each other and the species is that of color. Measurement of spores shows no appreciable differences; to attempt comparative measurements of other parts seemed irrelevant in view of their variability. It is to be regretted that in the present study no measurements of such properties as respiration could be made; other interesting and significant effects might have been found.

As the work proceeded it seemed desirable to give some attention to the cytology of the various forms obtained. Very little work has been done here. Indeed, Wakayama (**10**) seems to be the only one who has made any extensive studies of the cytology of *Aspergillus*. He found that two chromosomes were present and described briefly the process of division of the nucleus in the sterigmata. The results of the present study are substantially in agreement with those of Wakayama.

To obtain material suitable for cytological study, spores were planted on agar and the cultures allowed to grow until mature spores began to show at their centers. Each culture was then cut into several sectors; and these were lifted from the agar and dropped into the fixing fluid. Several different fluids were used, including chromic acid-acetic acid mixtures, Bouin's fluid, absolute alcohol-acetic acid, and others. If the sector of mycelium did not sink immediately into the fixing fluid it was first put into warm water and pumped, until sufficient gas had been removed to cause sinking. It was then transferred to the fixing fluid. After fixing, the sectors were washed and embedded in paraffin. The

thickness of most of the sections was 5 μ; of a few, 7 μ. The principal stain used was Heidenhain's iron-alum-haematoxylin, without counterstain. Other stains were used, but they showed nothing more than did the haematoxylin. The principal results

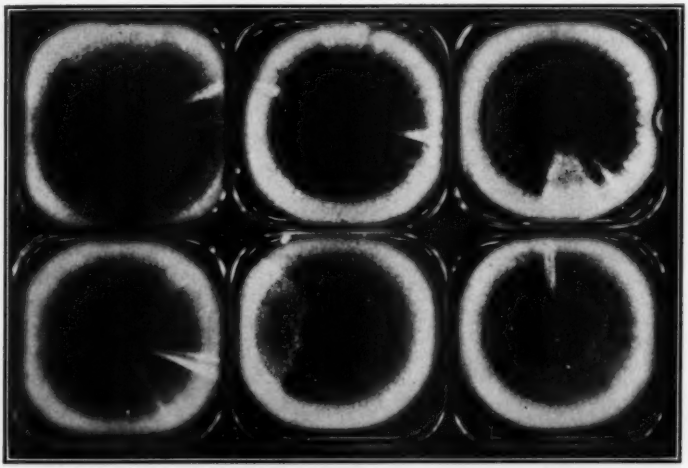


FIG. 3.

found in this cytological study are shown in figure 4, all the parts of which are drawn to the same scale.

Examination was confined mainly to the development of the spore-bearing parts, or sterigmata. When first formed these are minute spherical bodies thickly studding the entire surface of the vesicle. Each of these receives a single nucleus and elongates rapidly. There then occurs a nuclear division, various stages of which are shown in figure 4*a* and in the two right-hand parts of 4*b*. No significance is to be attached to the distinctly different sizes of the primary sterigmata at the time of nuclear division, nor does there seem to be any definite position for the nucleus. The two resulting nuclei always appear approximately at opposite ends of the sterigmata. The outer part of the primary sterigma is cut off to form the secondary sterigma, a condition which was frequently observed in its early stages. (The three left-hand parts

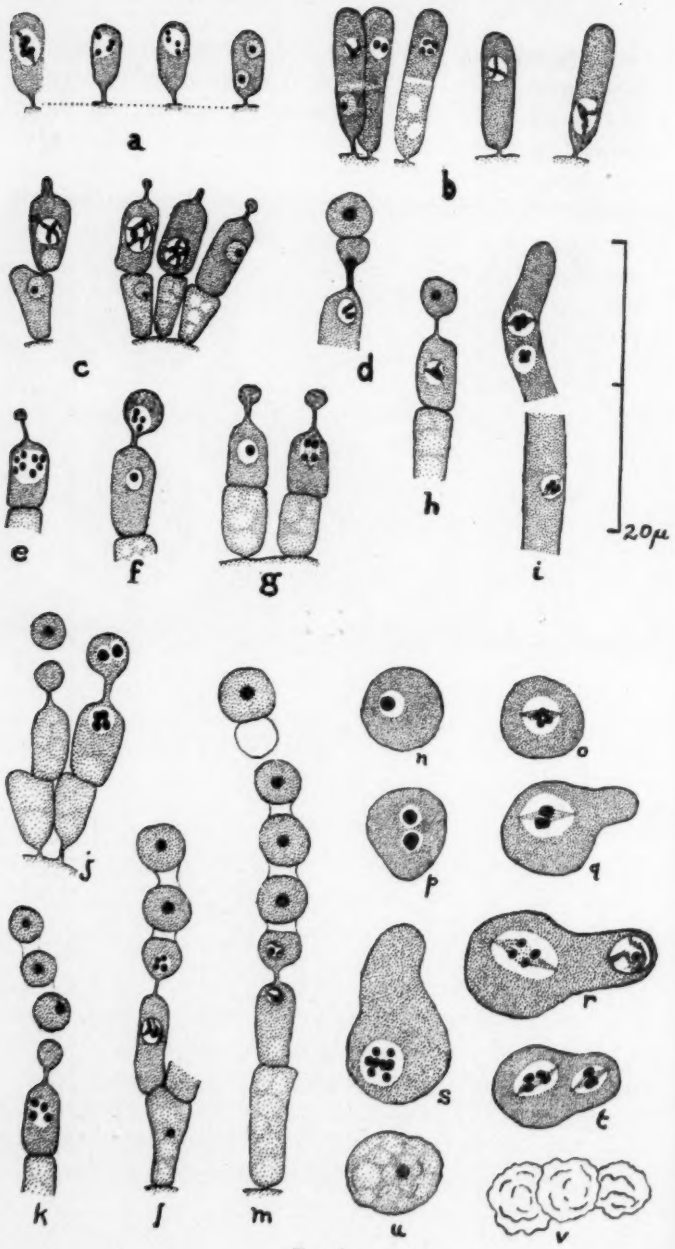


FIG. 4.

of FIG. 4*b*.) The nucleus in the secondary sterigma divides at once, often starting to divide before the division of the sterigma itself is complete. Figure 4*c* further shows stages in the division of the nucleus and also the initial stages of spore formation. At the left is illustrated what is probably the initial stage in the development of the second of the two secondary sterigmata commonly present on the primary sterigma of this species of *Aspergillus*. Figure 4*d* shows the apical portion of a secondary sterigma, from which one spore is already cut off, and another is forming and receiving a nucleus from the sterigma, while the remaining nucleus in the sterigma is already well advanced in another division. Very few cases were found in which a sterigma of the "giant" form had the chromosomes sufficiently separated to be distinguished. Examination of these indicates that in this form the number is twice that found in the species and in all the other variants (FIG. 4*e*). Nearly all the sterigmata of the "giant" form examined had the minute chromosomes so compactly massed that it was impossible to determine the number. Quite often the nucleus divides (FIG. 4*f*, *l*, *m*) after it has passed into the immature spore and an immature spore is binucleate (FIG. 4*j*). Since all the mature spores of this species seem to be uninucleate it may with reason be asked whether such a binucleate immature spore divides or whether the second nucleus has some other fate. The present study unfortunately yielded no information on this point. Figures 4*g*, *h*, *k*, *l* and *m* show additional stages of spore formation and nuclear division.

Divisions of the nuclei in the tips of the hyphae were frequently observed. In all these, two chromosomes were found (FIG. 4*i*). Except when these chromosomes were separating in anaphase, they were so close together that it was impossible to make out any details clearly. In no case was it possible to determine the number of chromosomes present in nuclei dividing in the tips of the mycelium of the "giant" form.

Because of the irregular ratio of development of the spores which were irradiated at 12 kv., it seemed desirable to attempt to examine them cytologically. To do this they were allowed to grow seven hours. Then, by pressing a fine needle gently against the surface of the agar, a few of the spores were pushed down into

the agar slightly. Small blocks of the agar into which spores had thus been pressed were cut out and treated in the same way as the material described above. Hundreds of spores were thus embedded, sectioned and studied. Some of these are shown in figures 4*n* to *v*.

At the time of fixation many of the spores had swollen considerably but still contained only one nucleus which showed no sign of dividing (FIG. 4*n*); other spores swollen about as much contained a dividing nucleus, with two chromosomes present (FIG. 4*o*). Such swollen spores soon became binucleate and at the same time showed the first indication of germ tube development as a small bulge from the surface of the spore (FIG. 4*p*). Frequently the development of the germ tube was well advanced before nuclear division in the spore was completed (FIG. 4*q*). As germination of the spores proceeded and the germ-tube elongated, divisions of the nuclei followed one another in rapid succession (FIG. 4*r, t*), each nucleus showing quite distinctly its two chromosomes, especially when the latter separated in pairs during anaphase. Of all the spores examined, only two were found to differ; in these the number of chromosomes present in the dividing nucleus was found to be twice the normal (FIG. 4*s*).

It is possible that these two spores showed the condition which led to the formation of the "giant" variant. In what way the doubling may have taken place is not apparent. Normally the growing spores had a uniformly dense homogeneous cytoplasm. In contrast to the normal spores, there were many in which the cytoplasm was extremely vacuolate (FIG. 4*u*). A few of these spores still contained a nucleus, which stained very faintly, but most of them showed no sign of any such body. Eventually these spores lost all their cytoplasmic content while at the same time the wall became very much wrinkled and the entire spore shriveled up (FIG. 4*v*). These spores apparently had gone through the preliminary stages of growth, swelling considerably, but never developed further.

The stages shown in figure 4 represent the principal phases in the development of the spores and the history of the division of the nuclei. Many additional stages were observed in all the vari-

ants described above, as well as in the species, but little else of significance was seen.

SUMMARY

Irradiation of spores of *Aspergillus niger* has produced several variant strains of this fungus. These strains differed markedly from the parent, most commonly in the color of the fruiting mycelium. One showed an evident increase in size. They have been continued through several asexual generations without showing any appreciable change. Calculations indicate that at the voltage used (ca. 12 electron kilovolts) the electrons penetrate the spores far enough to release most of their energy in the zone in which the nucleus is located. This suggests that the observed effects are a result of changes in the nucleus and that they may therefore be considered mutations. A cytological examination of the different variants showed only one evident nuclear change; in the large variant the number of chromosomes was twice that of the normal form. Otherwise the cytology of all the variants and of the species was alike. Several repetitions of the work gave comparable results, producing variant forms of apparently identical nature.

BIBLIOGRAPHY

1. **Cooper, F. S., C. E. Buchwald, C. P. Haskins & R. D. Evans.** 1939. Electron bombardment of biological materials. I. An electron tube for the production of homogeneous beams of cathode rays from one to fifteen kilovolts. Rev. Sci. Instr. **10**: 73–77.

   Haskins, C. P. 1938. The biological effects of low voltage cathode rays. Jour. App. Phys. **9**: 553–561.

   Whelden, R. M., C. E. Buchwald, F. S. Cooper & C. P. Haskins. 1940. Electron bombardment of biological materials. II. The rate of death of fungus spores bombarded in vacuum with cathode ray beams from 4 kv.–15 kv. Jour. Gen. Physiol. **23**: 391–400.

2. **Dickson, Hugh.** 1932. The effects of X-rays, ultraviolet light and heat in producing saltants in *Chaetomium cochliodes* and other fungi. Ann. Bot. **46**: 389–405.

3. **Dodge, B. O.** 1934. A lethal for ascus abortion in *Neurospora*. Science **79** (2052): 373–374.

   ——. 1935. A recessive factor lethal for ascospore formation in *Neurospora*. Bull. Torrey Club **62**: 117–128.

4. **Emmons, C. W. & Alexander Hollaender.** 1939. The action of ultraviolet radiation on dermatophytes. II. Mutations induced in cultures of dermatophytes by exposure of spores to monochromatic ultraviolet radiation. Am. Jour. Bot. **26**: 467–475.

5. **Haskins, C. P. & C. N. Moore.** 1934. X-ray and cathode ray tubes in the service of biology. Radiology **22**: 330–333.
6. **Holweck, F. & A. Lacassagne.** 1930. Action sur les levures des rayons X mous (K du fer). Compt. Rend. Soc. Biol. (Paris) **103**: 60–62.

Nadson, G. A. & E. J. Rochlin. 1933. Über Radiumheferassen. (Zum Problem experimenteller Rassenbildung.) Arch. Mikrobiol. **4**: 189–208.

7. **Nadson, G. A. & G. S. Philippov.** 1925. Influence des rayons X sur la sexualité et la formation des mutantes chez les champignons inferieurs. Compt. Rend. Soc. Biol. (Paris) **93**: 473–475.
8. **Nadson, G. A. & G. S. Philippov.** 1928. De la formation de nouvelles races stables chez les champignons inferieurs sous l'influence des rayons X. Compt. Rend. Acad. Sci. (Paris) **186**: 1566–1568.
9. **Nadson, G. & G. S. Philippov.** 1931. De la formation de nouvelles races stables de microörganismes sous l'influence des rayons X. II. Description des races de *Sporobolomyces*. Compt. Rend. Acad. Sci. U. S. S. R. **1**: 1–7.
10. **Wakayama, K.** 1931. Contributions to the cytology of fungi. III. Chromosome number in *Aspergillus*. Cytologia **2**: 291–301.
11. **Whelden, R. M.** 1938. Changes observed in cultures of *Aspergillus niger* bombarded âs spores with low voltage cathode rays. Mycologia **30**: 265–268.
12. **Wyckoff, R. W. G. & T. M. Rivers.** 1931. The effect of X-rays, cathode and ultraviolet rays on yeast. Radiology **17**: 1171–1175.

EXPLANATION OF FIGURES

FIG. 1. Spores in the region of the edge of the radiation band, those above the line which indicates this edge were rayed, those below unrayed. The dosage in this case was heavy, the voltage fairly low.

FIG. 2. Radiation effect on spores receiving of electrons of 11.52 ekv. energy, showing great irregularity of germination at the right, compared with control spores at the left.

FIG. 3. Mature cultures from groups of ten spores or less, showing variant sectors. The sector (center of the lower row) shows the large form. The other sectors are conspicuous because of their light colors, contrasting sharply with the normal black *Aspergillus*.

FIG. 4. *a*, stages in division of nucleus in primary sterigmata; *b*, stages in division of nucleus in primary sterigmata (2 right hand); stages in division of secondary sterigmata (3 left hand); *c*, nuclear divisions and early stages of spore formation; *d*, apex of sterigma and spores, nucleus migrating and nucleus dividing; *e*, nucleus in sterigma of "giant strain"; *f*, division of nucleus in immature spore; *g*, anaphase condition of nuclear division in secondary sterigma; *h*, prophase condition of nuclear division in secondary sterigma; *i*, hyphal tips with dividing nuclei; *j*, immature spores, one of which contains two nuclei; *k*, chain of uninucleate spores attached to secondary sterigma in which the nucleus shows distinctly the two pairs of

chromosomes; *l*, *m*, chains of spores, in the youngest of which a nuclear division is taking place while the nucleus remaining in the secondary sterigma is also dividing; *n*, much swollen spore containing a single nucleus; *o*, much swollen spore with nucleus dividing, and showing two chromosomes; *p*, binucleate swollen spore with germ tube just starting; *q*, germinating spore containing a single dividing nucleus; *r*, *t*, germinating spores each with two dividing nuclei; *s*, germinating spores in which there is twice the usual number of chromosomes present; *u*, spores containing vacuolate cytoplasm and a faintly staining nucleus; *v*, spores with much wrinkled walls and containing no cytoplasm.

HETEROTHALLISM IN CERATOSTOMELLA MULTIANNULATA

Ross W. Davidson

A number of workers have reported a variety of reactions in attempts to determine sexual processes in species of *Ceratostomella* as was pointed out by Leach [1] in 1934. At the suggestion of Andrus, a similar study was made to determine whether *C. multiannulata* Hedgc. and Davidson was heterothallic. This study was referred to by Andrus in 1936 [2] but was never published in detail. The results which were from single-ascospore and single-conidium cultures may be of interest to other workers and are recorded here.

Nine single-conidium cultures of *C. multiannulata* were secured from a perithecium-producing colony. None of these cultures produced fertile perithecia, but upon making all possible crosses with them, one of them formed perithecia when crossed with each of the other eight. Eight single-ascospore cultures were then obtained, none of which formed fertile perithecia. Four of these formed fertile perithecia when crossed with any one of the other four. The results of all possible crosses of these single-ascospore and single-conidium cultures are shown in Table 1. These results show that *C. multiannulata* is heterothallic with two sex groups represented, one comprising isolates No. 9 from a conidium and Nos. 10, 11, 12 and 13 from ascospores, and the other group the rest of the single-spore isolates. All intra-group crosses produced no perithecia and all inter-group crosses produced perithecia in abundance along the line where the mycelium of + and — strains grew together.

A few abnormally long-beaked perithecia developed in some of these single-spore cultures, but none was found to contain ascospores. The beaks of these sterile perithecia ended in tufts of

[1] Leach, J. G. The production of perithecia in *Ceratostomella ips* Rumb. Phytopath. **24**: 1037–1040. 1934.

[2] Andrus, C. F. Cell relations in the perithecium of *Ceratostomella multiannulata*. Mycologia **28**: 133–153, 1936.

TABLE 1

ALL POSSIBLE PAIRINGS OF 9 SINGLE-CONIDIUM CULTURES AND 8 SINGLE-ASCOSPORE CULTURES OF *Ceratostomella multiannulata* HEDGCOCK AND DAVIDSON

+ indicates fertile perithecial production; −, no fertile perithecia.

		Single-conidium cultures									Single-ascospore cultures							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Single-conidium cultures	1	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	2	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	3	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	4	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	5	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	6	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	7	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	8	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	9	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+
Single-ascospore cultures	10	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+
	11	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+
	12	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+
	13	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+
	14	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	15	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	16	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
	17	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−

brown hyphae instead of the short hyaline bristles found on normal ones.

The fact that this species develops an abundance of perithecia in a few days makes it easy to demonstrate this heterothallic condition. However, it is possible that single-spore cultures from other mass isolates would not always react in the same way.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

WHITE PERITHECIA AND THE TAXONOMY OF HYPOMYCES IPOMOEAE

WILLIAM C. SNYDER

In the course of studying a *Fusarium* root and fruit rot of cucurbits (**4**), a number of isolates of the causal fungus, which agrees morphologically with the species *F. javanicum* Koord., were collected from infected plants (*Cucurbita maxima* Duchesne and *C. Pepo* L.), grown in various parts of California. Single spore cultures of each collection were tested for pathogenicity, and all were found highly pathogenic. It was observed that perithecial fundaments (primordia) appeared in most of these cultures after a period of about a month or more, and that in some cultures they were red and in others white. In no case, however, did any of these primordia develop into perithecia.

Following the lead of Zycha (**6**) and Dimock (**1**) who demonstrated heterothallism in *Hypomyces,* conidial suspensions were prepared from cultures bearing white primordia, and applied to those bearing red primordia, and vice versa. In less than a week the fundaments in the fertilized cultures were developing into perithecia. Of special interest, however, was the fact that the cultures bearing red primordia developed only red perithecia while those bearing white primordia developed only white perithecia. These results indicated first, that the fungus under consideration is heterothallic, and second, that perithecial color may be either red or white. In view of the emphasis given to the color of perithecia in taxonomic treatments of the Hypocreales, the latter point seemed sufficiently significant to merit further study.

Upon maturation of the red and white perithecia obtained by means of cross fertilization, the morphology of the two types was compared. Both were found to fall within the limits of *Hypomyces Ipomoeae* (Hals.) Wr. (**5**), one differing however in whiteness of perithecia and both in pathogenicity.

Single ascospore cultures were prepared from the red and from

the white perithecia, respectively. These developed into *Fusarium* colonies representative of the original isolates, and in inoculation tests were found to be pathogenic on cucurbits. Thus their identity with the original isolates was confirmed.

In the isolations from nature, the cultures with red primordia proved to have a sex-reaction opposite to those with white primordia. Therefore reciprocal crosses of these types gave fertile perithecia, the color of which was dependent upon the direction of the cross. Approximately half of the single ascospore cultures from such a cross, whether taken from red or from white perithecia, developed red fundaments and the remainder developed white fundaments. The sex-reaction, however, was inherited independently of primordial color, so that it was possible to obtain fertile matings between types having only red fundaments, and also with those having only white fundaments. Reciprocal crosses involving only the one type of primordium as to color yielded only one kind of perithecial color independently of the direction of the cross.

These results showed definitely that not only are the red and white perithecial types interfertile, but that the stable color of the primordium (and therefore of the perithecium) is inherited. No intermediate types of perithecial color have resulted in any of the crosses, nor has more than one type of primordium appeared in any one single spore culture. The additional facts that the progeny from both red and white perithecia are all highly pathogenic on squash, and that all have the same morphology demonstrate beyond question that only one fungus is involved. Single ascospore cultures of this fungus then belong to two sex-reaction groups and may be described as being self-sterile, inter-group fertile, intra-group sterile, and hermaphroditic.

Although it is known that changes in perithecial color take place on aging, such as the change from red to brown or white to honey color, here the two types have remained distinct throughout their development. It has not been possible to change the one type into the other by means of aging, nor by the application of acid or alkali to them.

The occurrence of both red and white perithecia in the perfect stage of one and the same fungus, is particularly significant in

respect to taxonomy. Color as a taxonomic character has been used extensively in the past. More recently the trend has been to discount the value of color as a taxonomic criterion and to emphasize morphology especially in the imperfect genus *Fusarium* (3). This trend, however, has not become so marked where perfect fungi are concerned. The finding reported here that in *H. Ipomoeae* perithecial color is inheritable and may be red or white (perhaps additional isolates of the fungus will reveal other types of perithecial color) emphasizes the hazard in using color as a taxonomic character, even in the fruiting structures of perfect fungi.

H. Ipomoeae is not uncommon in its occurrence on decaying plant tissues, especially in warm climates. Most frequently it is known only as a saprophyte. An account of the disease of cucurbits referred to here, caused by a form of the above fungus, will appear elsewhere.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA

LITERATURE CITED

1. **Dimock, A. W.** Observations on sexual relations in *Hypomyces Ipomoeae*. Mycologia **29**: 116–127. 1937.
2. **Saccardo, P. A.** Sylloge fungorum **24**: 675. 1926–28.
3. **Snyder, W. C.** Notes on *Fusaria* of the Section Martiella. Zentralbl. Bakt. II **91**: 163–184. 1934.
4. ——. A *Fusarium* foot rot of *Cucurbita*. Phytopathology **28**: 19 (Abstract). 1928.
5. **Wollenweber, H. W. & O. A. Reinking.** Die Fusarien. Berlin. 355 pp. 1935.
6. **Zycha, H.** Uber *Hypomyces rosellus*. Zentralbl. Bakt. II **92**: 449–458. 1935.

A NEW POWDERY MILDEW

FRED J. SEAVER

(WITH 1 FIGURE)

During the autumn of 1939 the writer, while wandering through the conservatories of The New York Botanical Garden in search of material for class study on the fungi, observed a powdery mildew in full fruit on a rather unusual host.

The host proved to be *Jaborosa integrifolia* Lam., an Argentine plant. On checking up the material it was found that this host had been brought to The New York Botanical Garden by John K. Small and Edward J. Alexander from Louisiana in 1930, where the South American host had become established as a weed. It belongs to the family Solanaceae.

Whether the fungus came with the host plant from South America, or was acquired after the host had been brought up here is impossible to know. Or, again, whether the fungus was acquired in the greenhouse, or came with the host from Louisiana is uncertain.

The fungus belongs to the genus *Uncinula*. No powdery mildew has ever been reported on this plant, and the fungus appears to be different from any that the writer has seen described. It is characterized by its exceedingly long appendages, four or five times as long as the diameter of the perithecium. Since the fungus appears to have been unrecorded heretofore, the writer ventures to offer it as a new species.

Uncinula Jaborosae sp. nov.

Myclium occurring on both sides of the leaf but more copious on the upper side forming more or less confluent patches; perithecia abundant usually thickly gregarious near the center of the mycelial patches, reaching a diameter of 120 μ; appendages usually not exceeding 25, reaching a length of 4–5 times the diameter of the perithecium, subhyaline or very faintly colored, with a definite

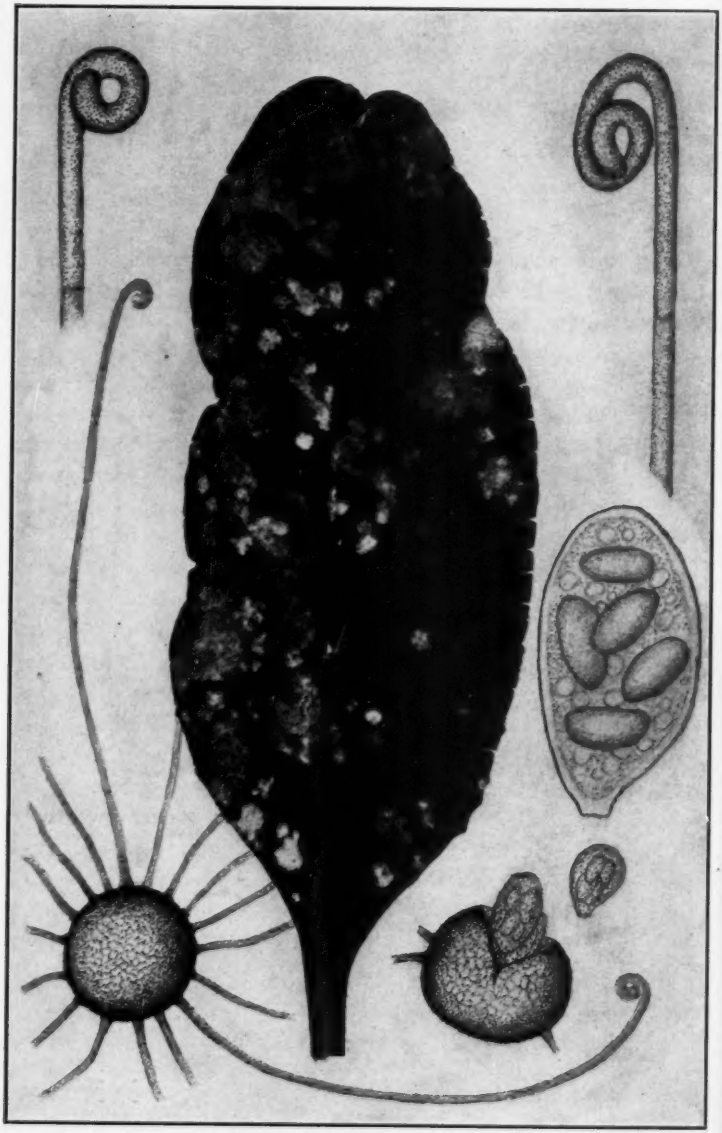


FIG. 1. *Uncinula Jaborosae.*

coil at the tip; asci several to each perithecium ellipsoid 46–50 × 65–70 μ; spores about 5 to each ascus, ellipsoid 10 × 20 μ.

On leaves of *Jaborosa integrifolia* Lam. in the greenhouse of The New York Botanical Garden, Dec. 15, 1939.

Amphigena; peritheciis sparsis vel gregariis, minutis, 120 μ diam.; appendicibus copiosis (15–25), longioribus (480–600 μ) subhyalinis; ascis 46–50 × 65–70 μ; sporis 5, ellipsoideis, 10 × 20 μ.

THE NEW YORK BOTANICAL GARDEN

MORENOELLA QUERCINA, CAUSE OF LEAF SPOT OF OAKS

E. S. LUTTRELL [1]

(WITH 13 FIGURES)

INTRODUCTION

A leaf spot disease caused by *Morenoella quercina* (Ellis & Martin) Theissen occurs on oaks within the Duke Forest. Although this disease is very common here, it has not previously been made the subject of special study. Furthermore, the developmental history of the pathogene is unknown and from a survey of mycological literature it is apparent that there is a scarcity of information regarding other related Microthyriaceae. These considerations led to a study of this disease and of the life history of its causal fungus, the results of which are presented in the following account.

SUSCEPTS AND RANGE OF THE DISEASE

Northern red oak, *Quercus borealis* Michx. var. *maxima* (Marsh) Ashe and black oak, *Q. velutina* Lam. are the principal suspects although the disease is frequently found on blackjack oak, *Q. marilandica* Muench., scarlet oak, *Q. coccinea* Muench., southern red oak, *Q. rubra* L., and willow oak, *Q. phellos* L. The comon white oaks, *Q. alba* L. and *Q. stellata* Wangenheim appear to be immune. Collections of *Morenoella quercina* in the Mycological Herbarium of the U. S. Department of Agriculture

[1] I wish by this means to thank Dr. Frederick A Wolf, Botany Department, Duke University, for suggesting the problem and for his advice and criticism during the course of the study and in the preparation of the manuscript.

I am also indebted to Dr. David H. Linder, Harvard University, and to Dr. William W. Diehl, U. S. Department of Agriculture, for information concerning collections of *Morenoella quercina* in the Farlow Herbarium and in the Mycological Herbarium of the U. S. Department of Agriculture, respectively.

and the Farlow Herbarium show that the fungus has been found elsewhere on the red oaks, *Q. rubra* L., *Q. pumila* Walter, and *Q. myrtifolia* Willdenow, and on the white oaks, *Q. geminata* Small, *Q. virginiana* Miller, *Q. virens* Aiton, *Q. minima* Small, *Q. Chapmani* Sargent, and *Q. stellata* Wangenheim. The fact that here *M. quercina* is restricted to red oaks while in other regions it has been found on white oaks as well, suggests the existence of different strains of the fungus capable of attacking the various species of oaks. The geographical range of *M. quercina* as shown by the above collections, is the southeastern United States for it has been collected in the District of Columbia, Virginia, North Carolina, South Carolina, Florida, Louisiana, Mississippi, and Texas.

APPEARANCE OF THE DISEASE

The disease is most conspicuous on seedlings and small trees, frequently involving a considerable percentage of their total leaf area. The first symptoms may be found in early summer when small blackened areas appear on the leaves. At this stage, unless examined microscopically, the lesions may be confused with injury produced by sucking insects. This leaf spot may be readily identified in any stage of its development, however, by the characteristic mycelium of *Morenoella quercina* on the upper leaf surface. Freehand sections cut parallel to the surface and examined under the microscope are the best means of determining this feature. The disease progresses slowly throughout the summer, but during September the spots rapidly increase in size until they reach diameters of a centimeter or more. On the upper surface they are roughly circular and are purplish black in color, while on the lower surface they appear as irregular, brownish discolored areas (FIG. 1). On severely infected leaves the spots become confluent and often cover almost the entire leaf. There is no premature defoliation. Nevertheless, the photosynthetic activity of the leaves is seriously impaired during a period in which normally food reserves are being accumulated in the stems and roots. A lowering of the vitality of seedlings as a result of severe infection for several successive seasons is the most serious consequence of the disease that could be expected. It is of no appreciable importance on older trees.

TAXONOMY OF THE PATHOGENE

The causal fungus is identical with *Aulographum quercinum* (also spelled *Aylographum* or *Ailographum*) which Ellis and Martin (**2**) described in 1883 on the leaves of *Quercus virens*. Tracy and Earle (**17**) transferred this species to the genus *Lembosia* in 1895 because the ascospores become dark brown at maturity. Both *Aulographum* and *Lembosia* were included in the

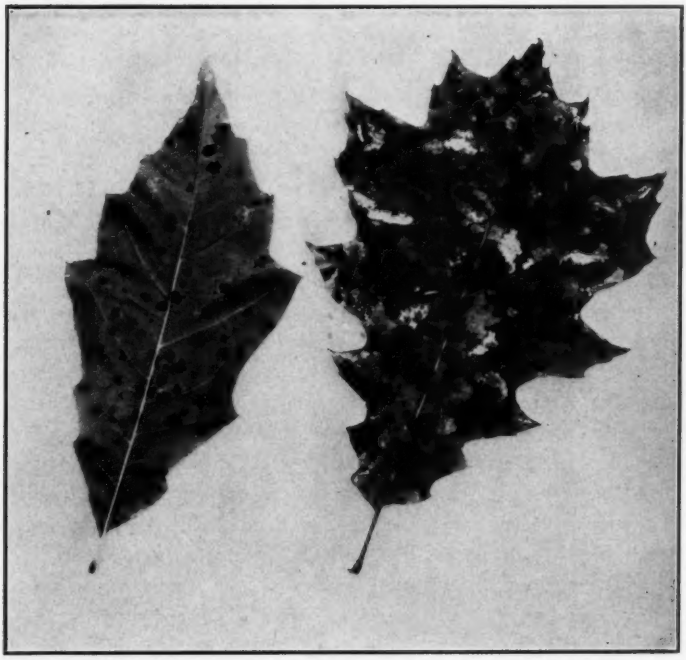


FIG. 1.

family Hysteriaceae, order Hysteriales. In 1883 Speggazini (**13**) created a new genus, *Morenoella*, and placed it in a new family which he called Hemihysteriaceae. Theissen (**14**) was able to find little difference between the genera *Morenoella* and *Lembosia* although they had been assigned to different families. He regarded both genera as having closest affinities with the Micro-

thyriaceae and, accordingly, in 1913 placed them in that family, maintaining them as separate genera, however, on the basis of the presence or absence of paraphyses. He emended the description of *Lembosia* to include only paraphysate forms, the forms lacking paraphyses being united under *Morenoella*. On this basis of separation *Lembosia quercina* (Ellis & Martin) Tracy & Earle became *Morenoella quercina* (Ellis & Martin) Theissen. In the same year Theissen (**15**) created the new family, Hemisphaeriaceae, which he united with the Microthyriaceae in a new order, the Hemisphaeriales. In 1917 (**16**) in a more extended treatment of the Hemisphaeriales, he expanded the order to include the Stigmateaceae, Polystomellaceae and Trichopeltaceae in addition to the two above-mentioned families. Members of Theissen's Polystomellaceae are included in theMicrothyriaceae by Arnaud (**1**) who removed this family to a separate order, the Microthyriales coordinate with the Hemisphaeriales.

STRUCTURE AND DEVELOPMENT OF THE PATHOGENE

In studying the development of the fungus only material from the leaves of *Quercus borealis maxima* was used. This was done to obviate confusion that might arise if different strains of the pathogene occur on different host species. Freehand cross sections and sections cut parallel to the surface were mounted in lactophenol in which 0.5 per cent cotton blue had been dissolved. By this means observations of the progressive development of the pathogene could be made frequently. To supplement these observations bits of infected leaf were fixed in acetic-alcohol solution at appropriate intervals, embedded in paraffin and sectioned at a thickness of 5 μ. The sections were stained with Haidenhain's iron alum haematoxylin and counterstained with Orange G in clove oil.

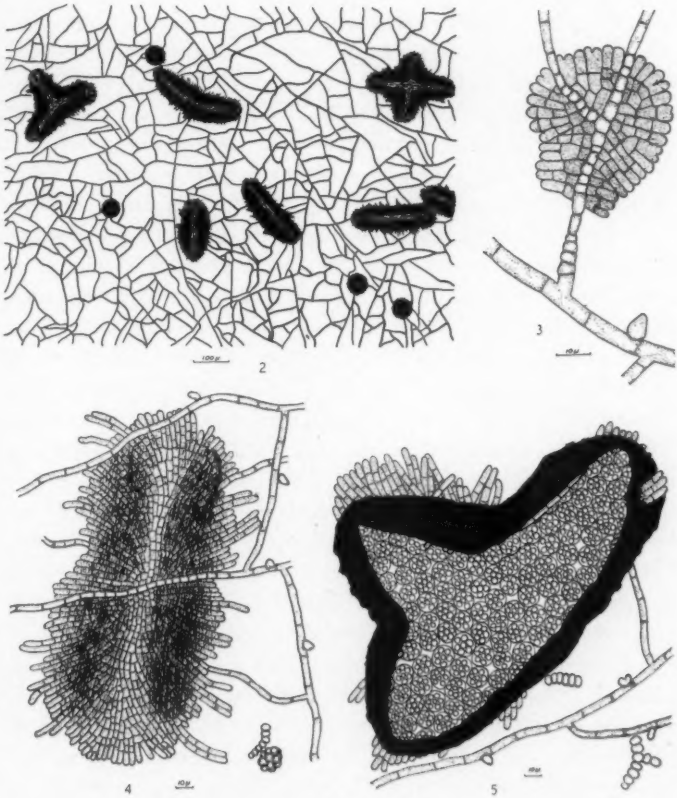
The Mycelium. The mycelium is restricted to the upper surface of the leaf and is entirely superficial, at least during the major portion of the summer. In cross sections of the infected leaf the hyphae are seen resting on the surface of the cuticle which appears to be intact and unaffected by the activity of the fungus. Nevertheless, the epidermal and mesophyll cells immediately beneath the hyphae are brown and disorganized. Ultimately the tissues ex-

tending from the upper to the lower leaf surface become necrotic. At this stage sections cut parallel to the surface show the mycelium radiating over the cuticle in all directions from the locus of infection. It is composed of dark brown thick-walled hyphae about 4.5 μ in diameter. Transverse septations divide the hyphae into cells 9–16 μ long. The hyphae branch and anastomose freely to form an open reticulum (FIG. 2). At intervals variously shaped ovoid to elliptical protuberances 6–9 μ long appear along the sides of the hyphae (FIGS. 3–5). These protuberances are the hyphopodia. Their presence together with the anastomosing habit of the brown hyphae gives a distinctive appearance to the mycelium. Frequently hyphal branches break up into short chains of large rounded cells or toruloid hyphal fragments. These become scattered over the leaf surface and may function as conidia. The rounded cells sometimes divide in several planes to form moruloid clusters (FIG. 4, 5).

In September hyaline hyphae appear beneath the cuticle. They are composed of large, irregular cells 3–10 μ in diameter. Each contains a peripherally disposed network of chromatic material (FIG. 9). In cross sections these cells appear as groups of large, spherical vesicles lying between the cuticle and the outer epidermal walls (FIGS. 6–8). They rapidly increase in number throughout the fall. In all sections examined, no morphologic connections between the external and the internal mycelium could be demonstrated. Nevertheless, the subcuticular mycelium is thought to be produced from the superficial mycelium because it develops only immediately beneath the latter.

The spermogonium. Spermogonia appear during August and are mature by the middle of September (FIG. 2). The mature spermogonium is hemispherical in shape with the flattened side appressed to the leaf surface. The wall is halved, consisting of a dome-like structure seated upon the leaf. A wall is lacking across the base of the spermogonium (FIG. 12). When observed in surface view the spermogonium appears to be made up of radiating hyphae although at maturity this feature is discernible with difficulty, except at the margins, because the wall becomes carbonaceous and opaque. At the center a pore or ostiole is formed by the separation of cells. In cross sections the upper wall

is seen to be composed of a single layer of darkened, thick-walled cells. This wall arches over a cavity whose floor is made up of a layer of large hyaline cells (FIG. 12). These cells are spermatiophores. Each cell is ampulliform in shape, the tapering apex



FIGS. 2–5.

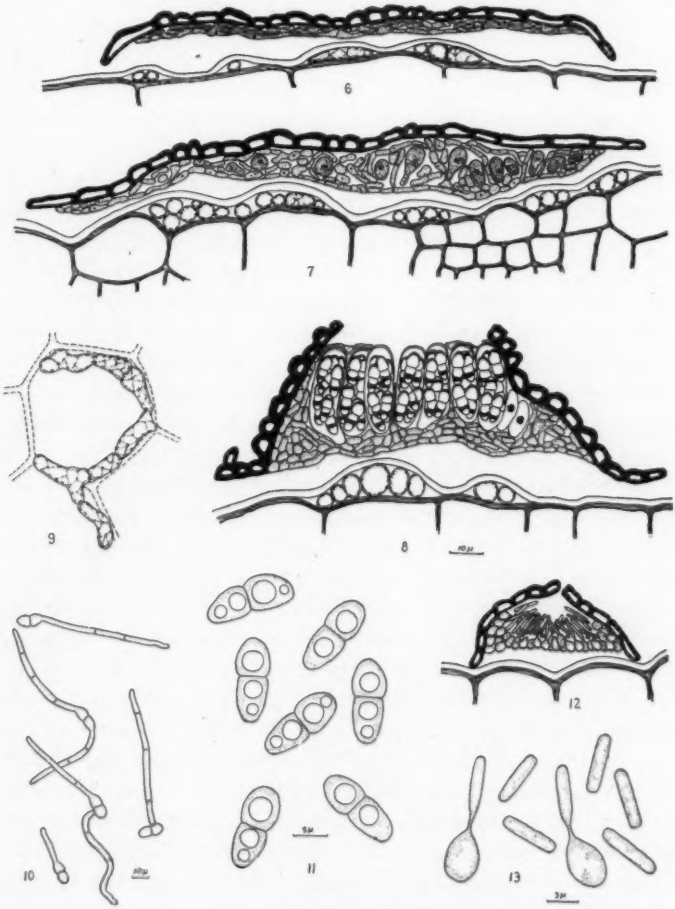
forming a sterigma, at the tip of which rod-shaped spermatia are abstricted singly (FIG. 13). Each spermatiophore probably produces several spermatia seriatim because the spermogonial cavity soon becomes filled with the bacilliform cells. The spermatia are hyaline and measure about $6.4 \times 1.3\,\mu$ (FIG. 13). They are

present from September throughout November being most abundant during October. After they are shed, the empty spermogonia remain in position until the leaves disintegrate.

The ascocarp. The formation of ascocarps is initiated while the leaves are still green and attached (FIG. 2). After abscission, an event which occurs about the end of November, the ascocarps continue development if the leaves fall in places which are not too dry. Early stages in ascocarp formation are found during September. Usually each ascocarp originates from a short segment of a single hypha. The segment becomes divided into a series of cells from the under side of which arises a group of hyphal branches that radiate in all directions over the surface of the leaf. They branch dichotomously and become fused laterally to form a flat, pseudoparenchymatous shield (FIG. 3). As a result of its origin from the under side of the hypha, this shield lies between the parent hypha and the leaf cuticle. The shield soon becomes black and carbonaceous so that its radiate structure can no longer be discerned except at the margins. Along its longitudinal axis a zone of lighter colored cells marks the line along which the shield is to split at maturity of the asci (FIG. 4). The shield varies in shape, being elliptical, Y-shaped, or in the form of a cross depending on whether the parent hypha was simple, branched, or crossed by another hypha at its point of origin (FIG. 2). During October hyaline branches originate beneath the shield and intertwine to form a compact plectenchymatous layer (FIG. 6). This layer extends beneath the entire shield but is only 2 to 3 cells in thickness.

Larger deeply staining hyphae, which are regarded as ascogenous hyphae, now appear within the plectenchymatous layer. It has been impossible to determine the origin of these hyphae. Terminal cells of branches of the ascogenous hyphae enlarge and become transformed directly into asci. The young asci are spherical and are scattered singly throughout the plectenchyma (FIG. 7). They are at first binucleate, but soon each contains only a single prominent fusion nucleus. Uninucleate asci may be found during January or sometimes as early as in December. The ascus elongates vertically and the fusion nucleus undergoes three successive divisions to form eight daughter nuclei. Around each of the daughter nuclei a membrane forms, delimiting the young asco-

spore. A large oil droplet forms in each end of the spore on either side of the nucleus. The nucleus divides and the resulting two nuclei move to opposite ends of the ascospore. A cross wall is



FIGS. 6–13.

next laid down dividing the spore into two cells. The nuclei become indistinct, the chromatic material appearing as a peripheral band near each end of the spore. The ascospores continue to en-

large at the expense of the epiplasm until they reach at maturity a size of 12–14 × 5–6 μ (FIG. 11). They are now obovate in outline with the anterior cell somewhat larger in diameter and more rounded than the posterior one. Ultimately they become dark brown in color. The ascospores are clustered within the ascus which is short, cylindrical and broadly rounded at the apex, and is about 27 × 10 μ in size (FIG. 8). Most of the asci within an individual ascocarp mature at the same time. The asci are originally separated by strands of plectenchyma but as they enlarge this tissue is crushed and disintegrated so that the mature asci stand side by side on a basal plectenchymatous layer 2 to 3 cells in thickness (FIG. 8). As a result of increase in size of the asci the shield is raised and split along its longitudinal axis and the torn edges are pushed back exposing the layer of asci.

If leaves that have lain on the ground are collected in early March and placed in a moist chamber, ascospores may be matured within a week or two. If, however, the leaves remain out of doors, ascospore formation begins towards the end of March. Mature ascospores are present from April until June.

Germination of ascospores and growth in culture. Horne (**7**) reported that he was unable to obtain germination of ascospores in his attempts to culture *Lembosia Rolfsii* Horne, a fungus closely related to *Morenoella quercina.* According to Fisher (**3**) only a single species of the Microthyriaceae (she does not indicate its specific identity) has been successfully grown in culture. *M. quercina,* however, was easily induced to grow on artificial media. Ascospores were obtained by suspending portions of leaves bearing mature ascocarps from the cover of a Petri dish so that the spores were shed upon agar or upon a slide placed in the bottom of the dish. The ascospores germinated within 12 hours on agar or in drops of distilled water placed on a slide or on the surface of an oak leaf. Germ tubes are produced either terminally or laterally from one or both cells of the ascospore. The walls are at first hyaline, but they soon turn light brown. The hyphae formed in culture are lighter in color and more irregular, and the hyphopodia are elongated and more pointed than in nature.

The fungus was grown on malt agar, on agar containing a decoction of oak leaves, and on synthetic agar. Although growth

was not very abundant, malt agar proved to be the most satisfactory medium tried. On this medium the fungus forms black, hemispherical colonies about a centimeter in diameter. Neither more extensive vegetative growth nor the formation of reproductive structures has been obtained in culture.

DISCUSSION

The superficial habit of *Morenoella quercina* immediately raises the problem of its nutritional relationships. During the summer the mycelium is restricted to the surface of the leaf and, as has been stated, there is no evidence of penetration of the cuticle. Nevertheless, it is evident that toxic substances are produced by the fungus and that they must diffuse through the cuticle into the leaf since the host tissue beneath the mycelium is killed. This being true, it is reasonable to assume that nutrients would likewise diffuse outward through the cuticle from the leaf cells to the mycelium. It is conceivable also that movement of food materials may be facilitated by alterations in the permeability of the cuticle which the fungus might produce without causing any apparent structural change.

Later in the season mycelium appears beneath the leaf cuticle. A parallel case is provided by the related *Lembosia Rolfsii* on vanilla in which Horne (**7**) found a subcuticular mycelium beneath the superficial mycelium. In both cases connections between the external and the internal mycelium have not been observed. On the other hand Arnaud (**1**) noted the presence of internal mycelium in the case of several other species of Microthyriaceae and has been able to demonstrate organic connections with the external mycelium. Such connections might be overlooked even in the examination of a large number of sections. It is concluded, therefore, that in *M. quercina* the subcuticular mycelium probably arises from the external mycelium and is connected with it. Evidence in support of this conclusion is afforded by the observation that the size of the lesions on oak leaves increases greatly in early September at a time coincident with the development of the subcuticular mycelium.

Morenoella quercina is a type of parasite which in its early development depends upon nutrients absorbed through the host

cuticle, but which later penetrates the cuticle and comes into more intimate contact with the host cells. There is, however, no evidence of penetration of the host cells by *M. quercina* although Arnaud (**1**) described the formation of haustoria by both the internal and external mycelia of many of the Microthyriaceae which he investigated. Maire (**9**) and Ward (**18**) have also shown that the mycelia of species of *Asterina* which remain superficial produce haustoria similar in structure to those of the Erysiphaceae.

Morenoella quercina is the second member of the Microthyriaceae in which the production of spermatia has been reported. Ward (**18**) previously described their formation in *Asterina spissa* Sydow. According to him erect clusters of delicate hyphae are produced on the mycelium and on the young ascocarps. Spindle shaped spermatia are abstricted from the tips of the erect hyphae. He was, however, in doubt as to the nature of these structures because of difficulty in determining their exact relation to the mycelium. Consequently it remains uncertain whether they actually constitute a stage in the life cycle of the *Asterina*. Although he made no mention of spermogonia in his text, Arnaud (**1**) figured, in a habit sketch of *Morenoella mollenideae* Arnaud, structures very similar to the spermogonia described for *M. quercina*. It seems highly probable that spermogonial stages will be found in the life cycles of other members of the Microthyriaceae when collections are made at the proper season. The question of their function in this group of fungi must, however, be left unanswered for the present. In view of the common occurrence of functional spermatia in other groups of Ascomycetes it seems likely that they are functional also in the Microthyriaceae but this assumption remains to be demonstrated.

The structure of the ascocarp of *Morenoella quercina* shows that it can be regarded neither as a perithecium nor as an apothecium. It is purely vegetative in origin; consequently it may be considered a stroma differentiated into a covering pseudoparenchymatous layer and an inner plectenchymatous portion within which the asci develop. Each ascus originates individually and by its expansion creates a locule within the stroma. The fungus tissue between the asci is ultimately crushed and absorbed by the developing asci so that they form a continuous layer within one common locule.

The ascocarp of the Microthyriaceae was formerly considered to be a halved perithecium. According to Gaillard (**4**) Raciborski (**11**), and von Höhnel (**5**) the shield is the lower half of the wall of an inverted perithecium, the morphological base being attached to the hypha above and the apical part having disappeared through reduction in response to the protection afforded by the host leaf. Consequently, the asci, which grow upward with respect to the surface of the leaf, were considered to be inverted with respect to the perithecium. The fruiting bodies of the Microthyriaceae have, therefore, been frequently referred to as inverted perithecia and for such structures the term thyriothecium has been applied. Von Höhnel (**6**) later came to the conclusion that this conception of their structure is false. He decided that the fruiting body was composed of two parts: (1) a sterile shield which developed from the lower side of the mycelium and (2) an erect perithecium which developed beneath the shield. The wall of the perithecium was greatly reduced as a result of the protection afforded by the shield. All of these investigators were concerned chiefly with the structure of the shield. Ryan (**12**) from the reports by Gaillard (**4**), Raciborski (**11**), and von Höhnel (**5**) on the Microthyriaceae and from her own observations on members of eleven genera of this family recognized the occurrence of four developmental types. According to her account the shield may originate either form: (1) any cell of the mycelium, (2) a large nodulose cell in the mycelium, (3) a short hyphal branch, or (4) a hyphopodium. Of these types the first is the most common and, as has been shown, is the one found in *Morenoella quercina.*

The most complete developmental study in the Microthyriaceae hitherto made is that of Ward (**18**) on *Asterina spissa.* Although it is difficult to make a comparison on the basis of Ward's description, the structural development in *Asterina spissa* seems to be similar to that in *M. quercina.*

Development in *Stigmatea Robertiana,* a member of the related family Stigmateaceae, is essentially the same as in *M. quercina.* According to Killian (**8**) its mycelium forms a continuous subculticular plectenchyma. Local thickenings in the plectenchyma, formed by the horizontal division of the cells, develop into stromata. Within the stroma the asci arise individually from the as-

cogenous hyphae. He was also able to determine that the ascogenous hyphae originate from an ascogonium after its fusion with an antheridium both of which organs are formed within the stroma.

Nannfeldt (**10**) has divided the Euascomycetes into three subdivisions based on the type of ascocarp; the Plectascales, the Ascohymeniales and the Ascoloculares. The Plectascales includes all Ascomycetes in which the reproductive organs are produced free on the mycelium and after fertilization become enveloped by a layer of hyphae to form a closed ascocarp. The ascocarp may or may not have a definite external wall and within it the asci are iregularly distributed. In the Ascohymeniales a hymenium composed of asci and true paraphyses is formed within ascocarps initiated as a result of a sexual stimulus. The ascocarps may be free or formed within a stroma and are either perithecia or apothecia. In the Ascoloculares, the reproductive organs are differentiated within a stroma which is vegetative in origin and the asci are formed individually in locules which they create by their growth within the stroma. Nannfeldt assigned the Hemisphaeriales to the Ascoloculares. The results of the present studies on *Morenoella quercina* and those ot Killian on *Stigmatea Robertiana* constitute evidence that these fungi should be included in the Ascoloculares. Additional and more complete developmental studies of other species are necessary, however, before any generalizations should be made for the Hemisphaeriales as the order is now constituted.

SUMMARY

A leaf spot disease of oaks caused by *Morenoella quercina* (Ellis & Martin) Theissen, present on oaks in the Duke Forest apparently occurs widely throughout the southeastern United States. This leafspot is found on the red oaks *Quercus borealis maxima, Q. velutina, Q. rubra, Q. coccinea, Q. marilandica,* and *Q. phellos* within the Duke Forest but does not infect the white oaks *Q. alba* and *Q. stellata.* Elsewhere it has been found on other oaks, both white and red. The disease causes a decrease in the photosynthetic activity of seedlings and young trees, lowering their vitality, but is of no appreciable importance on older trees.

Morenoella quercina is a superficial parasite which in its early development depends on nutrients absorbed through the intact host

cuticle. Later the hyphae penetrate the cuticle and form a subcuticular mycelium. The external mycelium sometimes produces toruloid hyphal fragments which may function as conidia.

M. quercina has a spermogonial stage which hitherto has not been described. Spermogonia and ascocarp initials are produced concurrently on the same external mycelium before the leaves are shed. The ascocarps continue development on the fallen leaves and reach maturity in spring.

The ascocarp is a flat stroma consisting of two parts: a pseudoparenchymatous, radiate shield which originates from a segment of a single hypha, and a plectenchymatous fertile layer which forms beneath the shield. The asci arise singly within the stroma. In their development the intervening sterile tissue is crushed and absorbed. The covering shield eventually becomes cleft to expose the mature asci.

Morenoella quercina belongs in the family Microthyriaceae, order Hemisphaeriales, and is included in the group Ascoloculares as delimited by Nannfeldt.

BIBLIOGRAPHY

1. **Arnaud, G.** 1918. Les Astérinées I. Ann. de l'École d'Agri. de Montpellier. II. **16**: 1–288.
2. **Ellis, J. B. & George Martin.** 1883. New Florida Fungi I. Am. Nat. **17**: 1283.
3. **Fisher, Eileen E.** 1939. A study of Australian "sooty moulds." Ann. Bot. II. **3**: 399–426.
4. **Gaillard, A.** 1893. Note sur les hyphopodies myceliennes et la formation des peritheces des *Asterina*. Bull. Soc. Myc. Fr. **9**: 95–97.
5. **Höhnel, F. von.** 1910. Fragmente Nr. 478 Mitteilungen X.
6. ——. 1917. Über die Perithecien der Microthyriaceen und die Gattung *Meliola* Fries. Ber. Deutsch. Bot. Ges. **35**: 698–702.
7. **Horne, William Titus.** 1905. A new species of *Lembosia*. Bull. Torrey Club **32**: 69–71.
8. **Killian, Ch.** 1922. Le développement du *Stigmatea Robertiana* Fries. Rev. Gén. Bot. **34**: 577–589.
9. **Maire, R.** 1908. Les sucoirs des *Meliola* et des *Asterina*. Ann. Myc. **6**: 124–128.
10. **Nannfeldt, J. A.** 1932. Studien uber die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. Nova Acta Regiae Soc. Sci. Upsal. IV. **8**^{2}: 1–368. (Vide p. 25–29.)
11. **Raciborski, M.** 1900. Parasitichen Algen und Pilze Javas, Batavia. III Teil, p. 43.

12. **Ryan, Ruth W.** 1926. The development of the perithecia in the Microthyriaceae and a comparison with *Meliola*. Mycologia **18**: 100–110.
13. **Speggazini, C.** 1883. Fungi Guaran. Pug. I, p. 258.
14. **Theissen, F.** 1913. *Lembosia* studien. Ann. Myc. **11**: 425–467.
15. ——. 1913. Hemisphaeriales. Ibid. **11**: 468–469.
16. —— **& H. Sydow.** 1917. Synoptische Tafeln. Ibid. **15**: 389–491.
17. **Tracy, S. M. & F. S. Earle.** 1895. Mississippi fungi. Miss. Exp. Sta. Bull. **34**: 80–122.
18. **Ward, H. M.** 1882. Researches on the morphology and life history of a tropical pyrenomcetous fungus. Quart. Jour. Micro. Sci. **22**: 347–354.

EXPLANATION OF ILLUSTRATIONS

FIG. 1. Northern red oak leaves bearing lesions produced by *Morenoella quercina*. (The leaf on the left viewed from the lower surface, the one on the right from above.); 2. Mycelium of *M. quercina* on the upper surface of lesion on oak leaf showing spermogonia and young ascocarps; 3. Surface view of young ascocarp showing the origin of the shield from a hyphal segment; 4. An ascocarp in a later stage of development; 5. A mature ascocarp; the shield has been cleft exposing the layer of asci. (Note hyphopodia on the mycelium in Figs. 3–5 and toruloid hyphae in Figs. 4 and 5.); 6. Cross section of a young ascocarp in which a plectenchymatous layer is forming beneath the shield; 7. Cross section of an ascocarp showing the young asci developing individually within the plectenchymatous layer; 8. Cross section of a mature ascocarp of *M. quercina*. (Hyphae of the internal mycelium are apparent between the cuticle and outer epidermal walls of the host cells in Figs. 6–8.); 9. Surface view of subcuticular mycelium; 10. Germinating ascospores; 11. Mature ascospores; 12. Cross section of a mature spermogonium of *M. quercina;* 13. Spermatiohpores and spermatia. Figs. 6, 7, 8, 9, and 12 drawn to scale indicated in Fig. 8.

A NEW SPECIES OF PISTILLARIA ON RICE STRAW

RUTH E. REMSBERG

(WITH 1 FIGURE)

An interesting fungus was found fruiting on rice straw from Louisiana, September, 1937. The straw had been placed in a moist chamber at room temperature. Abundant fruit bodies were observed within a week (FIG. 1). An examination of this fungus showed clearly that it belongs in the Clavariaceae. However, a search through the mycological literature on this family reveals no description to which it appears to conform.

Suggestions have been received regarding its disposition in the taxonomy of the fungi. Dr. G. W. Martin,[1] of the University of Iowa, suggests that it be placed in the genus *Ceratella*, while Dr. J. N. Couch,[2] of the University of North Carolina, thinks that it might be included in *Typhula*. A resumé of the taxonomic position of these two genera follows.

Quélet first recognized *Ceratella* as a distinct group of fungi (**6**), and made it a subdivision under the genus *Clavaria* to include those forms which were minute, unbranched and of a woody texture. Patouillard (**4**) elevated *Ceratella* to generic rank, using the characters, a pointed sterile apex and minute size, to distinguish it from other genera of the Clavariaceae. Quélet (**7**) retained *Ceratella* as a subdivision under *Pistillaria*, using the characters, an elongate clavula with a sterile pointed apex, to separate these species from the others. Saccardo (**9**) followed Quélet's example in disposing of these fungi. Patouillard (**5**) again preferred the generic rank, and used the fact that the hymenium surrounds only the middle portion of these minute plants as a basis for separation from the other genera of the Clavariaceae. He also mentioned that cystidia are present in the hymenial layer of *Ceratella*.

[1] Letter written March 20, 1938.
[2] Letter written December 24, 1938.

In later works on the Clavariaceae, *Ceratella* has been disposed of in various ways. Coker (2) does not recognize it as a genus. Bourdot and Galzin (1) follow Patouillard's example in giving it generic rank, while Killerman in Engler and Prantl (3) uses it as a subdivision under *Pistillaria*.

Although the fungus at hand frequently has a sterile pointed apex with the hymenium limited only to the middle portion of the plant, it does not seem wise to place it into *Ceratella*. The most obvious reason for excluding it from this genus is its large size. In all descriptions of *Ceratella* the species are minute, in fact, only a few millimeters tall. In addition, no cystidia are present in the hymenium of this fungus, while they are present in *Ceratella* (5). Furthermore, the position of *Ceratella* in the taxonomy is insecure, being accepted by some mycologists and rejected by others.

In my studies on the genus *Typhula* (8) I have proposed that all species having delicate, cartilaginous, club-shaped sporophores be placed either in *Typhula,* when a sclerotium is demonstrated to be present in the life history, or in *Pistillaria,* when a sclerotium is always lacking. The presence or absence of a true sclerotium seems to be the most reliable point of distinction between these two genera. Then all fleshy and large forms will be placed in *Clavaria*. With these facts in mind, I have placed this fungus in the genus *Pistillaria* where it may be included under the subdivision by those who wish to maintain the separation of those species with sterile tips.

Pistillaria Oryzae sp. nov.

Sclerotia absens. Sporophori orientes e mattis hypharum laxe implexarum, ramosarum, septatarum, multis confibulis praeditarum; filiformi, cylindrici, simplices v. ramosi, subulati, attenuati gradatim ab basi leviter crassa in acutissimum apicem, non diversi in distinctum stipitem et clavulam, leviter pubescentes ad basim; 25–80 mm. alti; toti albi, dein colorati (anglice ochraceous buff to pinkish cinnamon) ut senescunt aut arescunt; hymenium aut per totam superficiem sporophori praeterquam ultimum apicem extentum, aut tantum per mediam partem apice et basi sterilibus; compositi ex hyphis, densis parallelis, interdum cocleatim positis, subhymenio denso, centro composito ex hyphis laxe implexis, septatis, ramosis confibulatis, parvulis crystallis incrustatis. Basidia clavata, leviter attenuata ad apicem, quadrispora; 27.23–35.01 μ longa, 7.78–9.73 μ lata. Sterigmata recta v. leviter curva, attenuata in gracilissimum apicem; 3–4 μ longa, 1.5–3.0 μ lata ad basim. Basi-

diospori ovati v. fusiformi, cum manifesta guttula, hyalini, glabri, apiculo manifesto, truncato; 5.06–7.78 × 8.95–11.67 μ, modus 6.48 × 10.88 μ.

Hab. In foliis mortuis *Oryzae sativae*, saprophytica ut videtur.

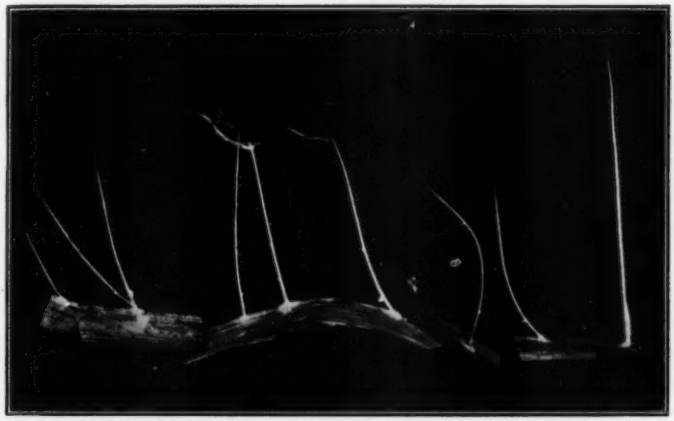


FIG. 1. Sporophores of *Pistillaria Oryzae* on dead leaves of *Oryza sativa*. ½ nat. size.

Sclerotia absent. Sporophores arising from mats of loosely interwoven, branched, septate hyphae with numerous clamp connections; filiform, cylindrical, simple or branched, awl-shape, tapering gradually from a slightly broadened base into a very acute apex, not differentiated into a distinct stipe and clavula, somewhat pubescent toward the base; 25–80 mm. tall; entirely white, becoming light ochraceous buff to pinkish cinnamon [3] with age or on drying; hymenium extending over either the entire surface of the sporophore except the extreme apex, or only over the middle portion the apex and base being sterile; composed of compact, parallel hyphae, sometimes spirally arranged, subhymenium compact, center composed of loosely interwoven, branched, septate hyphae with clamp connections, encrusted with small crystals. Basidia clavate, tapering slightly at the apex, 4-spored; 27.23–35.01 μ long, 7.78–9.73 μ broad. Sterigmata straight or slightly curved, tapering into a very delicate tip; 3–4 μ long, 1.5–3.0 μ wide at the base. Basidiospores ovoid to fusiform, with a prominent guttula, hyaline, smooth, apiculus prominent, truncate; 5.06–7.78 × 8.95–11.67 μ, ave. 6.48 × 10.88 μ.

[3] Ridgway, Robert. Color standards and color nomenclature. Washington, 1912.

HAB. On dead leaves of *Oryza sativa*, apparently saprophytic. Sporophores developed from leaves which had been placed in a moist chamber in the laboratory.

TYPE MATERIAL: *26861*, on rice straw, Herbarium, Department of Plant Pathology, Cornell University.

NOTES: The fungus was readily obtained in culture by fastening a sporophore in the lid of a petri dish and allowing the spores to be shot down onto agar in the bottom of the dish. The spores germinate within 24 hours, producing one or two germ tubes which rapidly elongate into septate, branched hyphae. The mycelial growth, which is very rapid in culture on potato dextrose agar and spreads in a characteristic fan-shape fashion, is abundant, white, aerial, and cottony. The fungus grows in culture from 6–33°C. or higher, with an optimum of 24–27° C. Fertile fruit bodies are produced abundantly in older cultures directly from the mycelium.

It was possible to obtain several crops of sporophores from the rice leaves in the moist chamber. After the first crop had matured, the leaves and chamber were placed in the refrigerator at 3° C. for 2–3 days and then removed to the laboratory. After 5–6 days new sporophores were produced. This was repeated five times, always obtaining 8–10 new sporophores.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, NEW YORK

LITERATURE CITED

1. **Bourdot, H. & Galzin, A.** Hymenomycetes de France, p. 1–761. 1927.
2. **Coker, W. C.** The Clavarias of the United States and Canada, p. 1–209. 1923.
3. **Killermann, S.** *In* Engler, A. & Prantl, K. Die natürlichen Pflanzenfamilien. 1928.
4. **Patouillard, N.** Les hymenomycetes d'Europe, p. I–XI + 1–162. 1887.
5. **Patouillard, N.** Essai taxonomique hymenomycetes, p. 44. 1900.
6. **Quélet, Lucien.** Enchiridion fungorum in Europa media et praesertim in Gallia vigentium, p. I–VII + 1–352. 1886.
7. **Quélet, Lucien.** Flore mycologique de la France, p. I–XVIII + 1–492. 1888.
8. **Remsberg, Ruth E.** Studies in the genus *Typhula* Fries. Mycologia **32**: 52–96. 1940.
9. **Saccardo, P.** Sylloge fungorum **6**: 758. 1888.

STUDIES ON HISTOPLASMA CAPSULATUM AND SIMILAR FORM-SPECIES. II. EFFECT OF TEMPERATURE [1]

ARDEN HOWELL, JR.[2]

(WITH 8 FIGURES)

In a preceding paper (3) the writer has shown that *Histoplasma capsulatum* Darling and *Sepedonium chrysospermum* (Bull.) Link resembled each other in their methods of spore formation and in the morphology of the aleuriospores, but that at the same time they differed in that *Sepedonium chrysospermum* produced phialospores, whereas *Histoplasma capsulatum* did not. Because of the fact that they seemed so strikingly alike on a morphological basis, and yet differed in the number of types of reproductive bodies formed, it became desirable to study the biology of these species. *Histoplasma capsulatum* Darling, which is parasitic on man, *Sepedonium chrysospermum* (Bull.) Link and *Stephanoma tetracoccum* van Zinderen-Bakker, which are parasitic on other fungi, and *Sepedonium xylogenum* Sacc., saprophytic on decayed wood were compared and differences in their responses to environment noted.

The first series of experiments is intended to determine the effect of temperature on the growth and sporulation of these fungi. In these experiments the fungi were grown on potato maltose agar made up according to the formula given in a previous paper (3). To insure uniformity of the medium, four liters of the potato decoction were made up at one time, and in order to obviate any

[1] Contribution No. 184 from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University. The material presented here and in a previous paper (3) is a portion of a thesis submitted to the faculty of Harvard University, June 1939, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biology.

[2] The author is deeply indebted to Dr. David H. Linder, under whom these investigations were conducted, for his constant advice and encouragement, and for his assistance in the preparation of the manuscript. He also expresses his gratitude to Dr. William H. Weston, Jr., for his many helpful suggestions and criticisms.

carry-over effects of the inoculum, the fungi were grown on the stock experimental medium at room temperature. Uniform blocks of the medium, approximately two millimeters in diameter, taken from the advancing edge of the colony, were used as inoculum. Three such blocks were inverted on equidistant points on each Petri dish, and three similar plates were used for each species at each

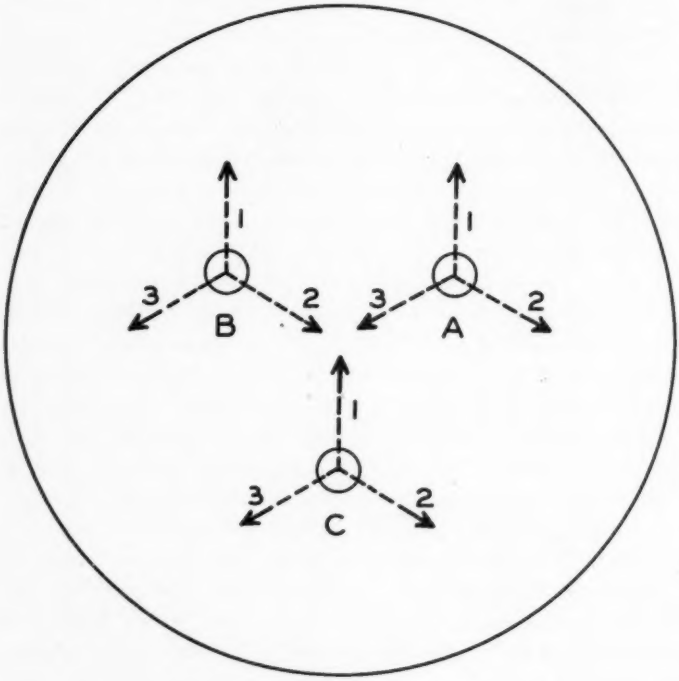


FIG. A.

temperature. The cultures were then placed at the various temperatures employed.

The diameters of the colonies were used as an indication of the amount of growth. These were measured in three directions on each colony and care was taken to measure the same diameters each time the measurements were made. This was accomplished by pasting small, circular pieces of paper, with arrows pointing in

TABLE I

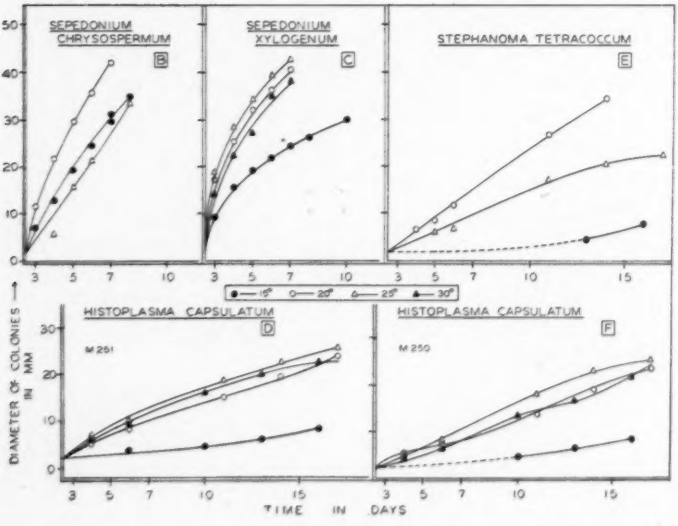
EFFECT OF TEMPERATURE ON GROWTH

Time in days	Temperature: 15° C.		20°		25°		30°		37°
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*
Sepedonium chrysospermum									
3	7.06	0.93	11.81	1.35	0.00		0.00		0 00
4	12.94	1.04	22.02	1.61	5.91	1.60	0.00		0.00
5	19.44	1.17	29.70	1.55	16.02	3.45	0.00		0.00
6	24.47	1.21	35.50	1.81	21.36	2.73	0.00		0.00
7	31.13	1.36	42.06	3.64	29.98	3.51	0.00		0.00
8	35.16	1.95	*		33.90	3.80	0.00		0.00
16	*		*		*		0.00		0.00
Sepedonium xylogenum									
3	9.41	2.59	17.63	2.38	18.55	1.37	15.01	1.60	0.00
4	15.85	2.06	25.52	1.31	28.57	1.61	22.37	2.98	0.00
5	19.24	2.03	32.08	1.72	34.12	1.84	27.22	3.04	0.00
6	21.82	1.99	36.61	2.27	39.42	2.37	35.04	2.03	0.00
7	24.57	1.76	40.46	3.79	42.55	3.14	38.39	2.60	0.00
8	26.41	1.42	*		*		*		0.00
10	30.30	3.33	*		*		*		0.00
16	*		*		*		*		
Stephanoma tetra-coccum									0.00
4	0.00		6.43	0.93	0.00		0.00		0.00
5	0.00		8.35	0.89	6.05	0.80	0.00		0.00
6	0.00		11.09	1.00	6.80	1.08	0.00		0.00
11	0.00		26.25	2.30	16.98	2.27	0.00		0.00
14	0.00		34.00	4.21	20.00	3.76	0.00		0.00
16	7.64	1.11	*				0.00		0.00
17			*		22.35	3.53	0.00		0.00
Histoplasma capsu-latum M 251									
3	0.00		0.00		4.13	1.14	0.00		0.00
4	0.00		6.01	0.45	6.44	0.14	6.09	0.94	0.00
6	0.00		8.35	0.62	9.98	0.13	9.33	1.55	0.00
10	4.97	0.64					15.59	1.41	0.00
11			15.10	0.73	18.62	0.90			0.00
13	6.62	0.52					20.06	1.38	0.00
14			19.50	0.61	22.48	1.66			0.00
16	8.43	0.10					22.33	2.23	0.00
17			24.00	0.69	25.11	1.65			0.00
Histoplasma capsu-latum M 250									
4	0.00		4.54	0.65	4.40	0.87	4.21	0 51	0 00
6	0 00		6 70	0.61	7.61	0.89	6.79	0.56	0.00
10	4.77	0.43					13.28	0.67	0.00
11			14.00	0.81	17.90	0.78			0.00
13	6.48	0.63					16.29	2.27	0.00
14			19.02	0.55	22.91	0.99			0.00
16	8.22	0.67					21.75	1.72	0.00
17			23.90	0.61	25.65	2.13			0.00

A—Average growth in millimeters based on 27 measurements.
B—Standard deviation.
*—Colonies have covered plates.

three directions, on the under side of each Petri dish (FIG. *A*). Each colony was designated by a letter, and the diameters measured along lines 1, 2, and 3. This has the advantage of constant measurements, but in spite of this degree of standardization, as the colonies increase in size and approach each other or the edge of the Petri dishes, there is evidence of pronounced inhibition of growth which results in shortening the radii at various points on the periphery of the colonies, a fact which makes the standard deviations so high (Table I).

The results obtained are shown in part in Table I and Figures *B–F*. A study of these results shows that the optimum temperature, under the conditions of this experiment, for *Sepedonium*



FIGS. *B–F*.

chrysospermum is 20° C.; that for *Sepedonium xylogenum* is 25° C.; that for *Stephanoma tetracoccum* is 20° C.; that for *Histoplasma capsulatum* is 25° C. whereas the maximum temperature at which growth occurs is between 25 and 30° C. for *Sepedonium chrysospermum* and *Stephanoma tetracoccum*, and lies between 30 and 37° C. for the remaining species. Although *Sepedonium*

xylogenum grew well at 30° C., its optimum was 25° C. Furthermore, it grew better at 20° than at 30° C. (FIG. *C, H*). On the other hand, *Histoplasma capsulatum,* for which the optimum temperature for growth was 25° C., grew almost as well at 30° and less rapidly at 20° C. It should be further pointed out that the growth curves of the two species of *Sepedonium* are somewhat similar (FIG. *B, C*) but in both cases are much greater than that of the two strains of *Histoplasma capsulatum* (FIG. *D, F*). *Stephanoma tetracoccum* takes a more or less intermediate position as regards rate of growth and shows more pronounced effects of temperature as may be determined from the fact that the growth curves are more distantly separated than in the other species (FIG. *E*). The two strains of *Histoplasma* are essentially identical in their rates of growth, both being extremely slow. It is also interesting to note that the rates of growth of *Histoplasma capsulatum* in this experiment are essentially similar to those reported by Moore (**4**) for another parasitic fungus in its saprophytic phase, *Endomyces capsulatus* Rewbridge, Dodge & Ayers (*Blastomyces dermatitidis* Gilchrist & Stokes, 1898[3]).

It should be emphasized, however, that the diameters of the colonies do not always indicate accurately the amount of growth of the fungi. This is very evident in *Sepedonium chrysospermum* (FIG. *B, G*) in which form the greatest radial growth at any time during the course of the experiment occurred at 20° C., the next greatest at 15° C., and the least at 25° C. However, the colonies at 15° C. were characterized by scanty aerial mycelium whereas at 25° there was produced a very dense, cottony, aerial growth, which more than compensated for the smaller radial spread (FIG. *G*). Were it not for the fact that at 20° C. the radial growth was greatest and sporulation earliest, the density of the mycelium of the colonies at 25° would lead one to suspect that 25° were the optimum, but in determining the optimum conditions for growth it seems to the writer that time of sporulation is an equally important factor, at least as regards the perpetuation of the species in nature. For example, at the end of eight days' growth, cultures of this species at 15° showed only a few phialophores, simple or with

[3] Benham, R. W.: The Fungi of Blastomycosis and Coccidioidal Granuloma. Arch. Derm. & Syph. **30**: 385–400. Sept. 1934.

but few branches, in a colony. After the same length of time, cultures maintained at 25° showed dense masses of well-developed phialophores. Likewise, cultures of this fungus maintained at 20°

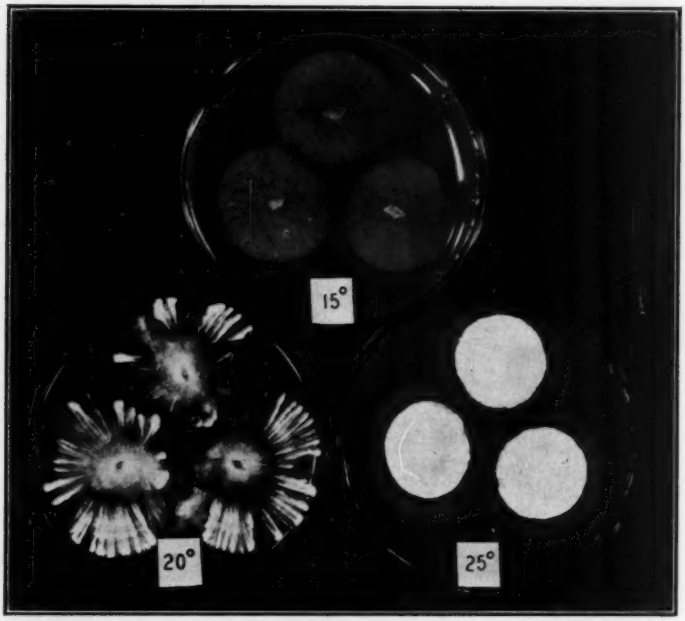


FIG. G. *Sepedonium chrysospermum* (Bull.) Link.

showed abundant phialophores and phialospores, but these were produced only on the radiate, aerial portions of the colonies (FIG. *G*).

Similarly, the aleuriospores, in cultures of *Sepedonium chrysospermum,* made their appearance first in those cultures grown at 20° C., and finally in those grown at 25°. Eventually, and only after a considerably longer period of growth, the greatest abundance of these spores was found in the cultures maintained at 25° and the least quantity in those at 15°. In contrast with this, aleuriospore production in *Sepedonium xylogenum, Stephanoma tetracoccum,* and *Histoplasma capsulatum* was correlated from the first with the abundance of the mycelium. This condition is

similar to that reported by Brown (1) for species of *Alternaria, Botrytis, Fusarium,* and other genera, in which he found that "with any particular medium the type of growth produced by the same fungus is very similar under the different conditions of the experiment, whether in air or in different concentrations of carbon dioxide, or whether at laboratory temperature or in cold store." In such cases measurements of the diameters of the colonies were good indications of the total amount of growth.

Because of the fact that in its parasitic phase *Histoplasma capsulatum* grows at body temperature or 37.5° C., it is interesting to note that in the series of experiments just described it failed to grow at 37° C. This led to an attempt by the author to restore the tolerance of this organism to this temperature. Accordingly, cultures of this species were started in 250 cc. Ehrlenmyer flasks containing either potato maltose agar or three per cent Difco Bacto-peptone agar or broth. The flasks with the solid medium and those with the liquid nutrient solution were placed at once at 30° C. After the cultures had grown three to four weeks, each series was divided, and one half placed at 32° C., the other half left at 30° C. Simultaneously, transfers were made from the cultures grown at 30° and also placed at 32° C. Each of these cultures grew well. At the end of two months, the procedure was repeated and the cultures which had been growing at 32° were placed at 34°, at which temperature each of the cultures gave excellent growth of the fungus. After the fungus had grown for three months at 34°, transfers were made to fresh media. Some of the cultures were placed at 34°, some at 37°, and one at room temperature. No growth occurred at either 34° or 37° even at the end of two months, but good colonies were produced in the flasks maintained at room temperature. It would appear from this that growth at a temperature of 34° is rather limited and that after a certain time the mycelium loses its ability to grow at the higher temperatures. Also it would seem that the spores, while viable, do not germinate readily, if at all, at 34° C. This is substantiated by preliminary experiments, which showed that although germination of ten weeks old spores took place readily at room temperatures, germination of three and five months old spores is erratic. Only once during these experiments was the writer successful in germinating spores

at 37°, and at that time only six per cent germination was obtained. Such erratic results substantiate the work of de Monbreun (2) who also reported difficulty in germinating the aleuriospores. While this appears to be true in cultures that have been maintained over

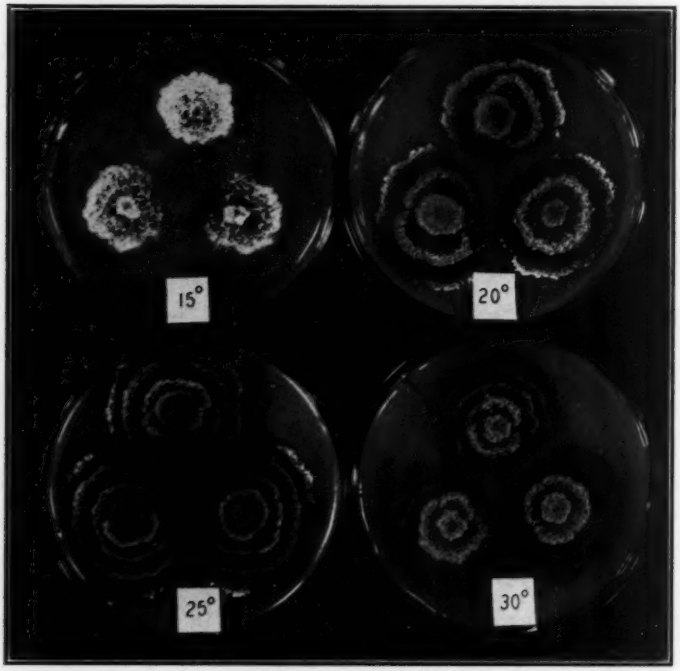


FIG. H. *Sepedonium xylogenum* Sacc.

a long period of time, nevertheless a more recently isolated strain [4] grew well at 37° C. on this medium. From these experiences it would appear first that the optimum temperature for the growth of the saprophytic phase of *Histoplasma capsulatum* is 25° C. Second, that through more frequent transfers and by growing this organism for longer periods of time at temperatures between 30 and 37° C., or, as shown by Weimer and Harter (5) in the case of

[4] This strain was recently isolated from a dog by de Monbreun to whom the writer is deeply indebted for his kindness in supplying the culture.

eleven species of *Rhizopus*, through the use of different media, it may be possible to restore the tolerance of the saprophytic phase of *Histoplasma capsulatum* to temperatures as high as 37° C. This supposition has been substantiated in recent work by successfully culturing the fungus (cultures M 250 and M 251) on freshly prepared blood agar at 37° C. In another respect the results of the present studies upon this parasitic fungus in its saprophytic phase are similar to those reported by Moore (**4**) who stated in his paper on another parasitic organism, *Endomyces capsulatus* Rewbridge, Dodge and Ayers: "These results seem to indicate that the optimum growth for this organism has been changed from approximately 37.5° C. in its parasitic condition to 25° C. in a saprophytic condition. This latter fact will be demonstrated in the animal inoculation experiments. It also shows that the longer an organism is kept in culture the greater will be its change in physiological phenomena to an optimum point for its changed environment." *Histoplasma capsulatum* appears to behave much in the same manner, and it is certainly clear that in its physiology as well as its morphology, its saprophytic and parasitic phases are distinct, and that the problem of the change of one phase into the other is in need of intensive study.

SUMMARY

The results of the investigations on the effect of temperature on the growth of *Histoplasma capsulatum*, *Sepedonium chrysospermum*, *S. xylogenum* and *Stephanoma tetracoccum* may be summarized as follows:

1. The optimum temperature for growth, all factors considered, was 25° C. for *H. capsulatum*, 20° C. for *S. chrysospermum*, 25° C. for *S. xylogenum*, and 20° C. for *Stephanoma tetracoccum*.

2. Sporulation in the case of *Sepedonium chrysospermum*, when grown on potato maltose agar, was greatest, not at temperatures in which maximum radial growth was produced, but at 25° C. Spore production in *Stephanoma tetracoccum*, *Histoplasma capsulatum* and *Sepedonium xylogenum* was correlated directly from the first with the abundance of mycelium. For these forms the optimum temperatures for sporulation were 20° and 25° C. respectively.

3. Attempts to restore the tolerance of *Histoplasma capsulatum* to body temperature (37.5° C.) were only partially successful.

DEPARTMENT OF BACTERIOLOGY,
DUKE UNIVERSITY,
DURHAM, NORTH CAROLINA

EXPLANATION OF FIGURES

FIG. *A*. Diagram to show method of measuring colonies; *B*, graph to show effect of temperature on growth of *Sepedonium chrysospermum; C*, graph to show effect of temperature on growth of *Sepedonium xylogenum; D*, graph to show effect of temperature on growth of *Histoplasma capsulatum* M. 251; *E*, graph to show effect of temperature on growth of *Stephanoma tetracoccum; F*, graph to show effect of temperature on growth of *Histoplasma capsulatum* M. 250.

FIG. *G*. 1–3. *Sepedonium chrysospermum* (Bull.) Link showing effect of temperature on growth at end of 8 days on potato maltose agar. 1, colonies at 15° C. show scattered aerial hyphae over the predominantly submerged mycelium; 2, colonies at 20° C. show radiate and rather dense aerial mycelium; 3, colonies at 25° C. show dense, cottony, aerial mycelium and a thin margin of submerged hyphae.

FIG. *H*. *Sepedonium xylogenium* Sacc. showing characteristic colonies after 8 days on potato maltose agar at various temperatures. Note the similar type of growth at all temperatures. Also, that there are zones in which aerial mycelium and aleuriospores are abundant. These alternate with zones in which aerial mycelium and aleuriospores are lacking. The number of zones varies with the temperature.

LITERATURE CITED

1. **Brown, W.** On the germination and growth of fungi at various temperatures and in various concentrations of oxygen and of carbon dioxide. Ann. Bot. **36**: 257–283. 1922.
2. **de Monbreun, W. A.** The cultivation and cultural characteristics of Darling's *Histoplasma capsulatum*. Am. Jour. Trop. Med. **14**: 93–124. 1934.
3. **Howell, A., Jr.** Studies on *Histoplasma capsulatum* and similar form species. I. Morphology and development. Mycologia **31**: 191–216. 1939.
4. **Moore, M.** A study of *Endomyces capsulatus* Rewbridge, Dodge and Ayers. A causative agent of cerebrospinal meningitis. Ann. Missouri Bot. Gard. **20**: 471–568. 1933.
5. **Weimer, L. & Harter, L. L.** Temperature relations of eleven species of *Rhizopus*. Jour. Agr. Res. **24**: 1–40. 1923.

NOTES AND BRIEF ARTICLES

Dodgea Malencon

As soon as the description and illustrations of this new genus came to hand, the writer was relatively certain it must be congeneric with *Truncocolumella* Zeller. Therefore, specimens of *T. citrina* Zeller were sent to Malençon to compare with the type of *Dodgea occidentalis* Malençon, and his reply to my inquiry is as follows (translated from French):

"I have examined with interest the specimens of *Truncocolumella citrina* which recently reached me in excellent condition. To my mind there is no doubt: your genus and mine are absolutely identical. I am entirely in agreement with you on this subject.

"On the other hand it is more difficult for me to say with certainty whether *Tr. citrina* and *Dodgea occidentalis* are specifically identical. *A priori,* I think that they are really the same species but there are certain macro- and microscopic differences between your specimens and Dodge's which permit the existence of some doubt.

"*Tr. citrina* is two to three times larger than *D. occidentalis.* Furthermore, your species is a clear yellow while Dodge's specimens were whitish. It is true that the latter had been preserved in alcohol for 17 years, a treatment which might have destroyed their original color. I described the species as whitish, but with reservations concerning the exact original tint, since Dodge furnished me no information on the subject; consequently I qualified my statement on the point (dfr. Bull. Soc. Myc. Fr. 44 (1938): 194. 1939; footnote No. 2).

"The anatomic structure of the two plants is in all respects entirely the same. There is, however, need to note that the spores of *Tr. citrina* are clearly shorter, more oval, and of more irregular shape than those of *D. occidentalis,* in which these bodies are long-elliptic, or sometimes subcylindric. . . . Taking into account the differences here described, especially with respect to the size of the carpophores and spores, I believe that it is at present more

prudent to conclude, *but only provisionally,* that *citrina* and *occidentalis* are two distinct but very closely related species. I am equally in accord with you on the subject of the priority of *Truncocolumella."*

Dodgea Malençon (Bull. Soc. Myc. Fr. **44** (1938): 193–194. 1939) was published March 31, 1939, while *Truncocolumella* Zeller (Mycologia **31**: 6. 1939) was issued February 1, 1939. *Dodgea* therefore becomes synonymous with *Truncocolumella,* and for *Dodgea occidentalis* Malençon a new combination is proposed, namely: **Truncocolumella occidentalis** (Malençon) Malençon & Zeller comb. nov.—S. M. ZELLER.